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INVESTIGATIONS ON THE STATISTICAL FREQUENCY DISTRIBUTION OF SOME PHYSICAL CHARACTERISTICS OF COTTONSEED KERNELS

S. HILAL AHMAD* AND MAZHAR M. QURASHI

Physical Research Division, Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi

(Received June 15, 1960)

Earlier measurements of the statistical distribution of the density of kernels of L.S.S. variety of cottonseed are extended to other varieties and also to cover other characteristics, namely, weight, oil content, and percentage oil content. The resulting distributions show an interesting bimodal character with 25% correlation on the average between weight and density. The percentage oil content distribution shows a $\pm 2\%$ variation about the mean and a possible correlation with the weight distribution.

In an earlier communication[1] dealing with the examination of possible nondestructive methods for the estimation of oil content of a single cottonseed, an exploratory study was made on the statistical distribution of the density of cottonseed kernels of the L.S.S. variety. Although it appeared that the measurement of density was not a suitable basis for the estimation of oil, its statistical distribution (Fig. 1, top) was remarkable in that it is distinctly bimodal, i.e. possesses two maxima. It was therefore considered worthwhile to extend this study to some other varieties and also to cover other characteristics of the kernels, such as weight, volume, oil content, etc.

1. Density Measurements on Kernels of Several Varieties

Because it had been found in the earlier measurements that even a sample of 25 kernels gave a fair indication of the phenomena being studied, it has been considered sufficient to use two lots of 25 seeds from each variety examined, and to estimate the standard deviations from the differences between the results for the two lots. Two random lots of 25 kernels were taken from each variety, only the obviously dried and shrivelled up kernels being rejected. The density was measured (as in the earlier work) with a pycknometer or specific gravity bottle, and the measurements were completed within a few days of dehulling; during this period the humidity ranged from 50% to 70%. A reproducibility of $\pm$ 0.3 mg. was attained in all the weighings, so that, for kernels with a mean weight of about 50 mg., the calculated values of specific gravity are reliable to $\pm 1\%$, i.e. to ± 0.01.

The results obtained with three varieties of Pakistani cottonseed, namely L.S.S., M4, and 4F, are plotted as frequency distributions in Fig. 1, using intervals of 0.05 in density. The short vertical lines through the plotted points represent the standard deviations as estimated from a comparison between the two lots of 25 kernels for each variety. In order to diminish the drawing errors, and to obtain smooth frequency distribution curves, it was found advisable to calculate the points near the middle with two sets of overlapping interval for the distributions, and this extra set of points is shown by hollow circles in Fig. 1. Using all the points thus plotted, very satisfactory curves can be drawn with an estimated error of less than ± 3 units on the percent frequency scale.

The graphs of Fig. 1 all show a more or less bimodal type of distribution. The higher of the two peaks, i.e. the main maximum, occurs at a density centred around 1.04 in the three varieties,

*Now at P.A.F. School, Korangi Creek, Karachi.

while the secondary maximum appears to vary considerably both in position and height, being most prominent in the case of L. S. S. cottonseed kernels. This provides some evidence for the probable existence of two types of seeds within the one variety (L.S.S.), which has been previously believed to show large unexplained variations in its oil content.[2] The distribution for L.S.S. is further seen (Fig. 1, top; broken lines) to be capable of decomposition into two mutually similar distributions centred about the density values 1.08 and 0.97, each with a half-value width of 0.08 units of density.

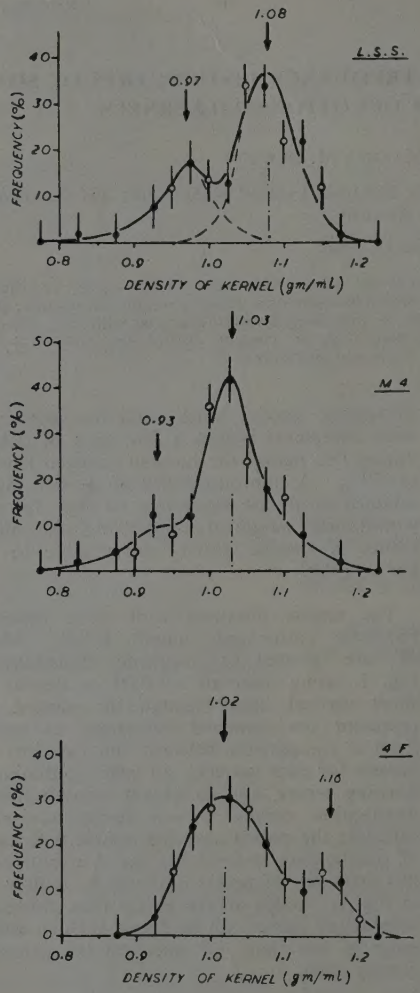

Fig.1.—Statistical frequency distribution graphs for density of kernels of three varieties of cottonseed, namely, L.S.S. (top), M4 (middle) and 4F (bottom). A density interval of 0.05 unit has been used (solid circles) and the hollow circles are intermediate points calculated for increased accuracy in drawing the graphs. The vertical lines through the points represent the estimated standard deviation.

2. Frequency Distribution Graphs for Weight of Kernel and their Correlation with Density

In an effort to interpret the above findings, similar distribution graphs of mass or weight have been plotted for the identical kernels used in the density measurements. Since the overall spread

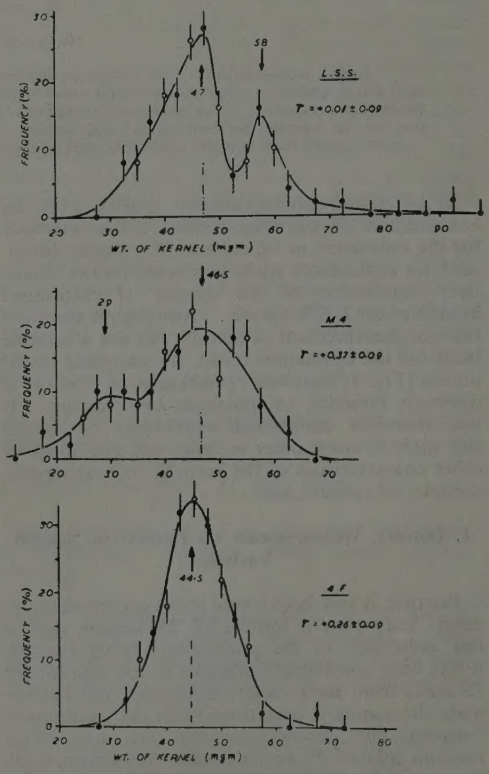

Fig. 2.—Corresponding frequency distribution graphs for weights of the kernels used in the density measurements for the three varieties: L.S.S. (top), M4 (middle) and 4F (bottom). The values of r, the correlation coefficient between weight and density, are given alongside the graphs.

of the weight for a kernel is roughly from 20 mg. to 90 mg., intervals of 5 mg. were chosen for calculating the distribution frequencies which are plotted in Fig. 2. As before, the short vertical lines indicate the estimated standard deviations, while the solid and hollow circles give the values calculated for two sets of overlapping intervals, which facilitate drawing of the smooth curves through the plotted points, giving an accuracy of $\pm 2\%$ frequency.

The distribution curve for the L.S.S. kernels is again found to possess a pronounced bimodal character with a deep minimum between the two peaks, while the graphs for the other varieties deviate but little from a simple nearly Gaussian distribution. Also, it is noteworthy that the major maximum occurs at almost the same weight for all the varieties, with a mean value of 46 $\pm$ 1 mg. The subsidiary maximum is, as before, variable in position as well as height.

Further light is thrown on the character of this subsidiary maximum in the graphs of Figs. 1 and 2 by calculating the coefficient of correlation 'r' between the data used for corresponding pairs of graphs. This was done by using the improved Spearman formula[3] for "Rank Correlation," viz.,

$$r = 1 - 6 \sum_{1}^{n} (x - y)^2 \Big/ (n^3 - n)$$

$$= 1 - \frac{6}{n} \sum_{1}^{n} (x - y)^2 \Big/ (n^2 - 1), \quad (1)$$

where 'x' and 'y' are the "ranks" of a given kernel with regard to the two properties, and 'n' is the total number of kernels examined. In order to obtain an estimate of the probable reliability of 'r', the total number of kernels of any one variety was (as before) divided into two groups of 25 each, and the value of 'r' was calculated separately for each group. In this way an overall standard deviation of ± 0.09 was derived and the mean values of 'r' calculated from the two groups are given as follows :

$$r_{L.S.S.} = +0.01 \pm 0.09$$
$$r_{M4} = +0.37 \pm 0.09$$
$$r_{4F} = +0.26 \pm 0.09$$

A notable feature is that all these correlation coefficients are positive, and if we make allowance for the indication for abnormal behaviour of the (distribution curves for the) L.S.S. variety by giving it one half the statistical weightage of the others, we get as the weighed mean* value for r,

$$r_{mean} = +0.25 \pm 0.06 \quad (2)$$

This shows that there is on the average 25% correlation of the high densities with the larger kernels.

*It is to be noted that the unweighed mean is not very different, being equal to + 0.21 $\pm$ 0.09.

3. Investigation of the Correlation between Peaks

Using this mean correlation coefficient as a reference, we can now examine the individual cases of the three varieties. Firstly, we find that the 4F kernels yield a value of r about equal to the mean, and correspondingly we find no subsidiary peak in the weight distribution, and only a small one in the density distribution. On the other hand, the value of 'r' deviates most from the mean value of +0.25 in the case of L.S.S. kernels, which accordingly show the most marked subsidiary maxima in the pair of distribution graphs. Secondly, it appears that (i) the large *negative* deviation (from r_{mean}) in the case of L.S.S. seeds corresponds to the subsidiary maxima occurring on *opposite* sides of the main maxima in the density and weight distributions, and (ii) the positive deviation from r_{mean} observed with M4 seeds is associated with these subsidiary peaks being on the *same* side of the main maxima in the two paired distributions. This suggests a strong positive correlation between the two properties of weight and density for the kernels corresponding to these subsidiary peaks. Numerically, the additional area under the subsidiary peaks in the case of L.S.S., is about 0.36 ± 0.02 of the total area under the distribution curve, while $(r_{mean} - r_{L.S.S.}) = 0.24$, thus indicating a (positive) correlation of $0.24/0.36 = 67\%$. This result also fits the distributions for M4 seeds, where the subsidiary peaks account for about 0.22 ± 0.09 of the area and for which $(r_{M4} - r_{mean}) = 0.12$. These data together lead to a mean figure of $60 \pm 6\%$ for the correlation between the pair of peaks. It is hoped to check on this conclusion by measurements on kernels of other varieties in future.

4. Statistical Distribution of Oil Content in L.S.S. Kernels

In the light of the above findings, an attempt was next made to examine the statistical distribution of oil in individual kernels of L.S.S. cottonseeds. For this purpose, we used the filter paper technique,[4] based on expression of the oil between a sandwich of filter papers, which has been developed for this very type of work. Each kernel was weighed, then placed in a sandwich of filter discs, two on either side, and the sandwich pressed at 7 tons between the plates of a 10-ton laboratory press. The area of the oil spot was determined

by measuring two mutually perpendicular diameters of the spot on each of two filter papers. The results obtained with 23 L.S.S. kernels are plotted as frequency distribution graphs for area of oil spot and weight of kernel, in Fig. 3 (top and middle, respectively) where the short vertical lines denote the estimated standard errors of the plotted points. While the mass distribution is very similar to the previously found bimodal one in Fig. 2, the distribution of area of oil spot, which is proportional to the total oil in the kernel, is strikingly different.

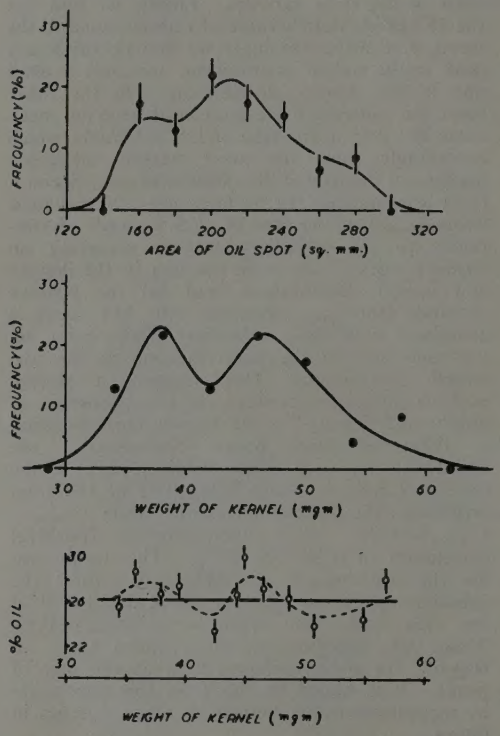

Fig. 3.—Graphs showing the frequency distribution of of (i) oil content measured as area of oil spot (top), and (ii) weight of kernel (middle), together with the percentage oil content against weight of kernel (bottom) for a sample of L.S.S. cottonseed kernels. The broken line shows the best curve through the plotted points in the bottom graph.

In an effort to elucidate this difference, the deduced percentage oil content (estimated[4] as 5.6 × area of spot/mass of kernel) has been plotted against the weight of the kernels in Fig. 3 (bottom), in which the points are means for two kernels, and the vertical lines indicate the standard deviation as estimated from the departure about these means. Although the broken line curve, which gives the best graph through the points, is seen to follow the general trend of the mass distribution curve in the middle of Fig. 3, it is noteworthy that the horizontal line drawn through the experimental points and shown by the full line is also a valid interpretation of the present data, because it is cut by 40% of the vertical lines and missed by 60%. It can therefore be concluded from Fig. 3 that (i) the variation in percentage oil content from kernel to kernel is at most of the order of ±2% on the weight of the kernel, and (ii) more accurate and extensive measurements are necessary before any correlations of percentage oil content with other constants of the seeds can be ascertained. Further studies in this direction are planned.

Acknowledgement.—The authors are grateful to Dr. M. Kamal, Mr. M.A. Ansari and members of the staff of the Cotton Research Institute for supplying samples of the different varieties of cottonseed and for useful discussions.

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2. Private communication from the Pakistan Cotton Committee.
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4. (a) S. N. Ahmad and M. M. Qurashi, Pakistan J. Sci. Ind. Research, **2**, 13 (1959).
   (b) M. M. Qurashi, S. N. Ahmad, S. A. K. Lodhi, ibid., **3**, 87 (1960).

BIOCHEMICAL AND NUTRITIONAL STUDIES ON EAST PAKISTAN FISH

Part IV.—Evaluation of the Mechanism of Fish Spoilage by Measurement of Tyrosine Values

M. QUDRAT-I-KHUDA, H.N. DE AND M.A.H. SHARIF

Food and Fruit Research Division, East Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Dacca

(Received July 10, 1960)

Eighteen species of muddy, shallow, and fresh river water fish grown in this region have been studied with respect to their spoilage characteristics by investigation of the change in the tyrosine values of their tissue during storage. The free tyrosine or similar compounds present in the fresh fish ranged from 28 to 106 mg. per 100 g. tissue. Storage for 24 hrs. at room temperature led to an increase of the value by 1 to 25 mg. % in case of muddy water fish and 48 to 120 mg. % in case of fresh river water fish. Among the fresh river water fish, the adults of smaller species showed a smaller increase of tyrosine values than the younger size of bigger species. The significance of this difference has been explained on the basis of the difference in the protein metabolism between the young and adult stages of an organism. Among the muddy water fish, those of non-air-breathing species behave similarly to the air-breathing species and this is due to similar ecological behaviour acquired by the non-air-breathing species while struggling for survival in the muddy shallow water.

1. Introduction

In the earlier communications of this series,[1,2,3] reporting the data on the assessment of spoilage of fish during storage under ordinary conditions at room temperature by measurement of iodine absorption, titratable acidity values and autodehydrogenase activities of several species of fish, it has been shown that the air-breathing fish like *Anabas testudineus* and *Heteropneustes fossilis* which generally thrive in shallow muddy water show some striking features with respect to their spoilage mechanism as compared to the non-air-breathing river fish like *Gadusia chapra, Mystus Aor, Labeo rohita etc.* This investigation has been continued further by a study of the change in the tyrosine values during storage of a large number of river and muddy water fish, and the present communication reports the data so obtained.

The technique of measurement of the tyrosine values by the Folin and Ciocalteu reagent has been adopted by Bradley and Bailey,[4] Beathy and Collins,[5] Tarr and Bailey,[6] Wood et al.,[7] Sigurdsson et al.,[8] Tarr[9] and others for the assessment of fish spoilage. This generally indicates the degree of proteolytic decomposition and represents not only the free tyrosine but also tryptophane, cysteine and other compounds containing -SH group, H_2S, etc., already present in the tissues and generated in larger quantity during spoilage. For convenience all the values are generally expressed as free tyrosine so as to correlate the spoilage with proteolytic decomposition.

2. Experimental Details

Eighteen species of fish grown in the muddy shallow water, in tank, or pond and in fresh river water have been investigated in the present study. In the muddy shallow tank, pond or 'beel' of this region, a large number of both air-breathing and non-air-breathing species of fish are grown even during the dry season of March to May. Six air-breathing species and four non-air-breathing species from muddy water have been studied in the present investigation. In fresh river water large varieties of both bigger and smaller species of fish are grown and in the present investigation four varieties of smaller species and three varieties of bigger species have been studied. In order to correlate the spoilage among the above species of varying sizes in a more precise manner, the investigation of the bigger species like *Labeo rohita, Labeo Calbasu* and *Mystus Aor* have been made on their younger samples not exceeding 10 inches in length so as to keep harmony with the size of smaller species of fish of muddy and fresh water groups.

Samples of fresh fish were collected from the market in the morning. The technique of the preparation of the samples and their storage in the room at ordinary temperatures of 80-84°F. was the same as was reported in the previous communications.[1-3] The tyrosine values for each sample was determined by the Folin and Ciocalteu method[10] by using 1 g. sample of the tissue for each determination. Along with the tyrosine values, the total nitrogen and moisture contents were simultaneously determined. Replicate determinations for each fish sample were carried out and the averages for these determinations for each sample are indicated in the tables.

On examining the data, it was observed that the change in the tyrosine values of the muddy water fish during storage showed distinctive features as compared to those of the fresh river water fish. Therefore, for convenience in the interpretation

of the results, the muddy pond water and fresh river water fish have been classed separately under groups A and B, respectively. The air-breathing and non-air-breathing fish of muddy water have further been subclassed under group A and bigger and smaller species of river water fish have been subclassed in group B.

3. Results and Discussion

The tyrosine value, total nitrogen and moisture percentages and the tyrosine nitrogen expressed as percent of total nitrogen in the fresh and stored fish samples are presented in Table 1. The increase in the above values due to storage are shown in Table 2.

Tyrosine Values of the Fresh Fish.—The results of Table 1 show that the tyrosine values in the fresh fish of group A ranged from 25 to 106 mg.% and those in the fresh fish of group B ranged from 28 to 106 mg. % which do not indicate any distinction between the above two groups of fish when fresh. When all these values are expressed as tyrosine then the nitrogen representing these values will amount only to 0.07 to 0.32% of the total nitrogen present in the tissues. This on storage showed an increase from 0.12 to 0.63% of the total nitrogen.

(*i*) *Change in the Tyrosine Values due to Storage.*—Storage for 24 hrs. led to an increase in the tyrosine values in all the species of both groups of the fish. From the data of Table 1 it is noted that in case of muddy water fish of group A the increase of the values ranged from 1 to 25 mg. per 100 mg. tissue and that of the fresh water fish of group B from 48 to 120 mg. per 100 mg. tissue. It thus seems evident that the muddy water fish, irrespective of their original tyrosine content, showed less increase of these values during storage as compared to the fresh water fish, whether of bigger or smaller

TABLE 1.—THE TYROSINE VALUES OF SOME SPECIES OF FRESH AND STORED FISH.

Name of the fish		Moisture %	Nitrogen %	Protein content %	Fresh fish at 0 hr.		Fish after 24-hrs.	
Zoological name	Local name				Tyrosine value mg./100 g.	Tyrosine nitrogen as % of total N	Tyrosine value in mg./100g.	Tyrosine nitrogen as % of total N
GROUP A: MUDDY WATER FISH								
(*a*) *Air-breathing fish*								
Ophicephalus striatus	Shol	76.3	2.65	16.56	50	0.1436	51	0.1462
Heteropneustes fossilis (*Block*)	Singi	76.74	2.50	15.64	43	0.1324	48	0.1479
Anabas testudineus (*Block*)	Koi	76.52	2.53	15.83	45	0.1374	50	0.1529
Clarias batrachus	Magur	79.1	2.80	17.35	42	0.1154	48	0.1320
Ophicephalus Punctatus	Taki	75.62	2.81	17.57	37	0.1014	45	0.1233
Ophicephalus marulius (*Hamilton*)	Gazar	80.04	2.68	16.75	37	0.1063	50	0.1436
(*b*) *Non-air-breathing fish*								
Nandus nandus	Meni	81.17	2.64	16.50	106	0.3081	111	0.3236
Mastacembelus panealus	Baine	74.70	2.78	17.25	35	0.0976	45	0.1255
Mystus vittatus	Tangra	80.02	2.28	14.25	87	0.2939	112	0.3767
Mystus corsula	Gulisha	73.65	2.78	17.37	45	0.1245	53	0.1469
GROUP B : FRESH FISH								
(*a*) *Small species*								
Ambassis nama	Chandkhira	70.0	2.83	17.68	28	0.0762	76	0.2067
Gadusia chapra	Chapila	71.20	2.78	17.38	37	0.1025	103	0.2853
Barbus (*puntius*) *sophore*	Puti	78.19	2.98	18.63	95	0.2472	160	0.4234
Mugil cascasia	Kachki	70.78	2.81	17.57	36	0.0886	147	0.4028
(*b*) *Big species*								
Catla catla	Katla (naola)	82.01	2.63	16.43	98	0.2869	204	0.5971
Labeo rohita	Rohu	76.7	2.61	16.31	95	0.2803	190	0.5607
Labeo calbasu	Kali bahus	75.03	2. 54	15.93	106	0.3314	210	0.6366
Mystus aor	Air	77.44	2.76	17.26	105	0.2929	225	0.6377

species. This independence of the above distinctive characteristics between the two groups of fish with respect to their original tyrosine values and size of the fish is clearly seen when the data of the muddy water fish *Nandus nandus* on the one hand and the small fresh water fish *Mugil cascasia*, on the other, are considered. *Nandus nandus* on an average is 6″ in length and originally has a tyrosine value of 106 mg. percent in the fresh tissue which on storage shows an increase of the above value by only 5 mg. *Mugil cascasia* with an average size of less than one inch contains only 36 mg. percent of tyrosine in the fresh condition but shows a very high increase by 111 mg. per 100 g. tissue on storage.

It thus appears that the spoilage is much more rapid in fresh water fish than in the muddy water group, irrespective of their original tyrosine values in the fresh condition.

TABLE 2.—INCREASE IN THE TYROSINE VALUES AND OF THE TYROSINE NITROGEN EXPRESSED AS PERCENTAGE OF TOTAL NITROGEN AFTER 24-HOUR STORAGE.

Name of the fish (zoological)	Increase in the tyrosine value in mg. per 100 g.	Increase in Tyrosine nitrogen as % of total nitrogen
GROUP A (MUDDY WATER)		
(*Air-breathing species*)		
Ophicephalus striatus ..	1	0.0026
Heteropneustes fossilis ..	5	0.0155
Anabas testudineus ..	5	0.0155
Clarias batrachus ..	6	0.0166
Ophicephalus punceatus ..	8	0.0219
Ophicephalus marulius ..	13	0.0373
(*Non-air-breathing species*)		
Nandus nandus ..	5	0.0155
Mastacembelus panculas	10	0.0279
Mystus vittatus ..	25	0.0828
Mystus corsula ..	8	0.0224
GROUP B: (FRESH WATER)		
(*Smaller species*)		
Ambassis nama ..	48	0.1305
Gadusia chapra ..	66	0.1828
Barbus (puntius) sophore	65	0.1762
Mugil cascasia ..	117	0.3142
(*Big species*)		
Catla catla ..	106	0.3102
Labeo ruhita ..	95	0.2804
Labeo calbasu ..	104	0.3052
Mystus aor ..	120	0.3348

(*ii*) *Spoilage Characteristics in Relation to Species Size.*—In case of river fish it has also been noted that smaller species of fish like *Barbus puntius*, *Ambassis nama*, *Gadusia chapra*, etc., show less increase in the tyrosine values as compared to the bigger species like *Labeorohita*, *Catla catla*, *Mystus aor* etc. It may be mentioned in this connection that the bigger species have been investigated in their younger stage up to a length of 10″ whereas the smaller species like *Barbus Puntius*, and *Gadusia chapra* were collected in their adult stage ranging in length from 3″ to 8″. The above difference in the increase in the tyrosine value due to difference in the size and species of the fresh water fish may be explained on the basis of the knowledge of protein biochemistry in the organism.

In any organism during the growth stage more protein remains in circulation in dynamic state for the formation of the new cells after fulfilling the requirement for the wear and tear of the old cells. But in the adult stage less amount of protein remains in circulation since only the maintenance phase for the wear and tear of the old cells, operate at that stage. Because of such difference in the protein metabolism between the young and adult stages of any organism it seems quite plausible that during young stage more tissue protein will be exposed to proteolysis whereas in adult stage less amount of protein will be subjected to decomposition. This explains the small increase of tyrosine values due to storage of adult fish of the smaller species like *Barbus puntius*, *Gadusia chapra* etc., as compared to the younger samples of the bigger species like *Labeo rohita*, *Catla catla* and *Mystus aor*.

(*iii*) *Spoilage in Relation to Ecological Behaviour.*—It has already been mentioned that the muddy water fish whether air-breathing or non-air-breathing, show less generation of tyrosine and like compounds during storage as compared to the fresh water fish whether bigger or smaller species. The non-air-breathing muddy water fish like *Nandus nandus*, etc., are physiologically similar to the fresh water smaller species fish like *Barbus puntius*, *Gadusia chapra*, etc., but in storage they

behave in a manner almost similar to air-breathing species like *Anabas testudineus*, *Hetropneustes fossilis* and *Clarius batrachus* with regard to this spoilage characteristics involving proteolytic decomposition. This may be explained as due to the fact that non-air-breathing fish like *Mystus vittatus*, *Mystus corsula*, etc., when grown in the muddy water along with the air-breathing species like *Anabus testudimus*, *Heteropneustes fossilis*, *Clarius batrachus etc.*, have also to struggle hard for their survival like the air-breathing ones. Thus, they acquire similar ecological behaviour for which they may also survive longer period like the air-breathing ones after they are caught, although physiologically they cannot be grouped together. It thus appears that ecological behaviour acquired during the struggle for survival may also play an important role on the spoilage mechanism of the fish tissue.

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FRUITS AND FRUIT PRODUCTS

Part VI.—Evaluation of Certain Seasonal Fruits of East Pakistan for Industrial Uses

M. QUDRAT-I-KHUDA, B.D. MUKHERJEE, S.F. RUBBI AND N.A. KHAN

Food and Fruit Research Division, East Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Dacca

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Twenty-four varieties of fruits of East Pakistan have been studied. Percentages of proteins, fats, fibre, reducing sugars, pentose, pectin, vitamin C, and inorganic elements such as calcium, sodium and potassium have been determined. Experiments have also been performed to assess their suitability for juices, syrups, jams, jellys, morabbas, haluas and other valuable food products.

1. Introduction

In East Pakistan fruits grow in different seasons. The harvest periods in some cases are very short with variable crop yields. The result is that there is often a glut in the market and consequent deterioration and wastage. In order to prevent such happenings, there should be attempts to handle the fruits in a proper way for storage as well as to preserve them by some means or the other. Such processes demand full knowledge of these fruits in all respects, botanical, chemical and biological. We collected the fruits as the seasons came, selected varieties from a fruit and analysed them according to the standard procedures or procedures modified to suit the purpose. Many fruits of importance have thus been previously subjected to critical studies.[1-5] The present paper relates to another series of fruits.

The varieties in size and quality of the fruit gave somewhat average values characteristic of each fruit. The quality of the fruit was not only judged by the visual characteristics of colour, appearance and maturity, but also by actual tasting. The organoleptic response from different persons made such evaluations easier. Some twenty-four fruits from the Dacca markets were used for analysis during the last half-year period. The analysis was carried out in a very methodical way in all cases, so that the changes in fruits were kept at a minimum.

2. Experiments and Discussion

The fruits are collected from the local markets as the seasons come and their edible portions are subjected to analysis as fresh as possible. Protein, fat, fibre, reducing sugar and pentose have been determined according to the methods of A. O. A. C.[6] Pectin has been estimated by the modified method of Carre and Haynes.[7] For the estimation of vitamin C, titrimetric method[8] has been followed. As for the analysis of inorganic elements, iron and phosphorus have been estimated colorimetrically,[9] calcium volumetrically[10] and sodium and potassium gravimetrically.[11]

As to the proximate analysis , moisture content in a fruit is quite high, it invariably falls in the range of 80-90% with a few in the range 70-80% In the latter, we got the only fruit called 'bael,' both green and ripe. From Table 1, it is clear that other important constituents, viz., protein, fat, fibre, reducing sugar and pectin ranges

TABLE 2.—ANALYSIS OF THE ASH OF THE FRUITS (CALCULATED ON ASH BASIS).

Serial No. of fruits of Table 1	Ash *	Na_2O	K_3O	CaO	P_2O_5	Fe_2O_3
1	2.11	Trace	36.50	11.26	9.76	1.08
2	5.32	—	—	2.89	7.31	0.43
3	7.91	0.51	38.04	9.37	8.10	0.12
4	5.83	0.41	37.5	4.56	3.92	0.29
5	3.10	0.11	66.77	7.89	11.48	1.38
6	4.01	—	—	14.36	7.40	0.72
7	2.23	0.90	80.7	13.18	8.20	3.04
8	2.92	1.11	38.7	5.75	8.59	3.42
9	4.41	1.21	33.33	6.98	12.97	1.42
10	2.22	0.11	45.04	19.50	20.63	0.63
11	7.20	2.11	30.89	3.08	4.88	0.40
12	4.15	0.12	49.21	1.40	3.75	0.61
13	5.98	0.41	38.97	0.92	3.98	0.52
14	8.50	0.23	48.31	4.20	8.39	0.80
15	1.35	0.15	53.10	3.01	6.21	0.68
16	3.90	2.19	39. 82	2.95	10.61	0.33
17	3.92	2.08	51.82	2.08	6.80	0.48
18	6.05	3.10	55.78	11.63	7.01	0.12
19	4.50	1.33	55.68	1.21	9.99	0.03
20	4.00	14.48	15.32	5.60	8.01	0.14
21	5.00	0.95	48.81	8.01	12.24	0.03
22	3.72	1.52	57.91	13.54	12.91	0.17
23	3.21	10.95	14.02	5.06	14.22	0.04
24	2.44	1.09	51.29	13.05	16.98	0.45

*Calculated on dry weight basis of original fruits.

from 2.1 to 18.0%, 1.0 to 10.11%, 2.3 to 22.8%, 2.4 to 65.1 % and 9.5 to 46.2 %, respectively. Vitamin C content varies from 508 to 41 mg. %. Table 2 represents the mineral contents of the fruits calculated on ash basis. It is apparent that potassium, calcium and phosphorus contents of all fruits are good, and vary from 14.0 to 80.7%, 12 to 19.5%, and 3.9 to 20.6%, respectively; but sodium and iron contents are low and vary from 0.12 to 14.48% and 0.03 to 3.42%, respectively. Of the nutrients, vitamin C draws our particular attention. It was very carefully determined, vitamin C contents of amlaki, green papaya, pineaple, pomelo, limes (kagazis) and oranges are very noteworthy. The spectacular findings relate to amlaki which has the highest quantities of vitamin C. Our experiments over a period of four years established the seasonal variation of this fruit in vitamin C contents contaminated by tannins. Considerable efforts in such a period resulted in successful removal of tannins by three agents, gelatin, resin and also pectinaceous materials.[12] Moreover, in several experiments we removed just the astringent quantity of tannins and concentrated the juice for various types of syrup making.

Our determinations indicated loss of vitamin C but we kept them to a minimum. Concentrates in syrups[13] with as high as 450-680 mg./per cent were also obtained. The beverages[14] were also enriched in vatimin C by such extracts. The residual pulp was used for morabba, halua and other valuable products. The seed oil was also isolated and found to be good for soap-making. The pectin, and acid contents in the fruits[8-13] (Table 1) gave us clues for jam and morabba making. The jams[15] of papaya, bael, sapeta, guava, kadbael were profitably made. The morabba of green papaya, bael and pomelo peels, and amlaki were quite luscious. One outstanding

TABLE 1.—PROXIMATE ANALYSIS OF THE SEASONAL FRUITS (CALCULATED ON DRY WEIGHT BASIS).

Local name	Botanical name	Water g.	Ash g.	Protein g.	Fat g.	Fibre g.	Reducing sugar g.	Acida g.	Pectin g.	Pentose g.	Vit. Cb mg.
1. Amlaki	*Phyllanthus emblica*	82.10	2.11	2.82	4.71	14.02	30.52	14.92	11.52	8.61	508
2. Karamja	*Carassa carandas*	89.33	5.32	10.52	6.37	11.22	25.11	6.31	9.51	8.42	28.11
3. Kancha pepa	*Carica papaya*	91.01	7.91	12.41	2.43	13.21	42.12	1.19	21.52	9.13	30.21
4. Paka pepa	,,	88.94	5.83	7.93	2.61	5.74	25.8	1.12	46.21	5.01	20.81
5. Kui	*Zigyphus guguba*	86.02	3.10	11.10	2.52	5.52	12 .01	3.36	13.01	4.10	5.12
6. Anarash	*Ananas sativum*	90.15	4.01	3.42	2.01	13.02	30.21	9.31	—	5.31	80.42
7. Kancha bael	*Aegle mermelos*	65.02	2.23	5.34	1.42	—	5.11	2.10	—	6.01	—
8. Paka bael	,,	67.03	2.92	11.44	6.35	—	11.10	2.91	—	7.24	7.10
9. Jambura	*Citrus decumana*	85.04	4.41	3.72	5.54	5.21	40.22	—	23.31	4.01	87.12
10. Kamala	*Citrus aurantium*	87.01	2.22	9.15	2.12	4.15	33.12	—	12.71	4.31	50.12
11. Kad Bael	*Feronia elephantum*	74.10	7.20	13.20	2.28	19.64	30.44	0.52	0.84	7.64	15.32
12. Desi plum	*Zigyphus guguba*	78.32	4.15	4.55	1.80	6.15	34.05	0.50	1.55	9.05	—
13. Mesta	*Hibiscus sabdariffa*	84.23	5.98		1.80	8.28	2.46	1.38	trace	20.52	13.12
14. Chalta	*Dillenia Indica*	77.92	8.50	2.10	1.05	9.05	15.05	0.05	0.50	10.05	17.95
15. Sapta	*Achras sapata.*	79.61	6.35	14.05	0.90	16.10	57.10	1.00	0.65	3.60	12.91
16. Sharifa	*Anona squamora*	86.62	3.90	4.86	1.02	5.22	65.10	trace	trace	2.10	10.48
17. Guava	*Psidium guavam*	75.41	3.92	2.08	1.52	22.88	16.48	0.60	4.82	3.92	10.99
18. Jalpai	*Fr olive*	82.52	6.05	18.05	2.55	14.10	10.05	0.10	1.60	10.25	9.33
19. Jamrul	*Chrysaphyllum cuenito*	90.00	4.50	10.11	9.90	8.92	49.01	16.00	—	—	6.80
20. Musk melon	*Cucum is melo-*	92.72	4.00	14.10	3.91	9.71	23.22	—	trace	—	4.03
21, Amra	*Spondias mangifera*	87.00	5.00	7.91	10.11	19.12	23.42	—	trace	—	8.81
22. Dumar	*Ficus glomerata*	85.00	3.72	5.98	6.00	4.92	9.00	—	—	—	11.50
23. Latkau	*Baccaurea sapida*	91.12	3.21	5.12	3.14	2.33	65.21	—	—	—	8.36
24. Gab	*Diaspyros empryopteris*	75.01	2.44	3.88	4.08	12.84	28.80	trace	—	—	19.20

[a] Calculated as citric acid. [b] Calculated as mg. per 100 g. of the edible portion.

feature of roselle (mesta) is that its extracts contain both acid and pectin and hence addition of sugar and proper boiling gave good jelly. Guava jelly was also prepared from the pulp freed of seeds, fibre and peels with addition of sugar and acid. Similar products were also obtained from pineaple, kadbael, and sapeta by addition of pectin, sugar and acid, as they contain not enough pectin. Such addition, according to composition, may give various types of delicious products of fruit that will keep for a long time and at the same time eliminate the wastage of fruits.

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TABLE 3.—SOME SPECIFIC CHARACTERISTICS OF FRUITS AND THEIR SOURCES.

Sl. No. of fruits of Table 1	Total wt. of the fruits g.	Peel %	Edible portion %	Seed %	Colour		Acceptability as food	Popularity	Availability & statistics		
					Green	Ripe			Season	Approx. amount	Locality
1	105	0	70	30	Light green	Light green	+++	+++	Winter	Abundant.	E. Pak. (all over)
2	250	0	100	—	„	Yellow	+++	+++	Winter.	„	„
3	750	7	91	2	Deep green	Green with yellow	+++	+++	Whole year.		
4	755	9	90	3			+++	+++	„		
5	10		60	40	Green	Yellow	+++	+++	„		
6	1500	10	90	—	Green	Brown	+++	+++	„		
7	800	10	90	—	Green	Yellow			Winter		
8	800	10	90	—	—	—	+++	+++	Summer		
9	2500	12	86	2	Green	Yellow	+++	+++	Winter		
10	200	5	94	1	Green	—	+++	+++	Winter	Small quantities	Sylhet Chittagong Hill Tracts
11	435	42.52 (sheel)	50.57	6.71	Dark green	Dark brown	+	+	Rainy season	Abundant.	Chitg. Dacca
12	5.9	21.23	38.41	40.35	Green	Red	++++	++++	Winter	„	E. Pak.
13	10.12	—	49.40 (calyx)	50.59 red.	Light red	Scarlet red	+++	++++	„	small quantities.	E. Pak.
14	1200	15.79	59.2 9	25.00 (seed + mucilage + seed cover)	Light green	Yellowish green.	+++	+++	Winter	Appreciable quantities.	E. Pak.
15	74.32	13.44	80.73	5.85	—	Dark red	++++	++++	—	Small quantities	Chitg. Jessore Rajshahi
16	190	22.10	71.05	8.40	Green	Brownish green	++++	++++	Winter.	„	„
17	105	18.09	78.09	3.80	Green	Yellow	++++	++++	Rainy season	Abundant	E. Pak.
18	12.82	2.0	5.62	5.2	Deep green	Light green	++++	++++	Winter.	Small quant.	E. Pak.
19	30.9	17.54	81.96	0.50	Milk white	Yellowish white	++	++	„	„	E. Pak.
20	840	5.71	93.41	.88	Light green.	Yellowish green	++++	++++	Summer	Abundant.	E. Pak.
21	100	2.1	40.0	57.9	Green	Yellow	+++	+++	„	Appreciable quant.	E. Pak.
22	9.80	24.48	55.08	21.44	Green	Reddish green	+	+	„	Small quant.	E. Pak.
23	12.20	4.30	4.30	1.60	Green	Ghee-yellow	++	++	„	„	E. Pak.
24	120.12	15.5	79.0	5.5	Green	Brown	++	++	Winter	„	E. Pak.

\+ Very slight +++ Moderate
++ Slight ++++ Very much

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IN VITRO I^{131} UPTAKE BY TISSUES OF RAINBOW TROUT

M. Maqsood,* E.P. Reineke and P.O. Fromm

(with the technical assistance of H.W. Kaczkofsky)

Department of Physiology and Pharmacology, Michigan State University. East Lansing, Mich., U.S.A.

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The data on the *in vitro* I^{131} uptake by different tissues of rat indicate that iodide concentration mechanism in mammary, salivary and gastric glands but not in the liver or placenta appears to be similar to that of thyroid gland.[1,2] In the case of fish, review of published results indicates that the thyroid hormone is probably not involved in the regulation of oxidative metabolism in fish[3,4] and as such it was decided to study the *in vitro* I^{131} uptake by different tissues of rainbow trout (*Salmo gairdnerii*) with a view to find out whether any relationship exists between the cold and warm blooded animals so far as the concentration of iodide in different tissues is concerned.

The results reported by a number of workers on the *in vivo* effects of various thyroidal and antithyroid agents on the thyroid gland of fish are not in general agreement (reviewed by Pickford and Atz).[3] Fromm and Reineke[5] reported that peak thyroidal accumulation of intraperitoneally injected I^{131} in intact rainbow trout occurred four days after injection. Hoffert and Fromm[6] in a similar experiment showed that peak uptake occurred 24 hours after injection, the discrepancy between the two experiments being due to incomplete correction for body background activity in the earlier work. Using radioiodine, Baker[7,8,9] has shown that heterotopic thyroid tissue is present in platyfish, a guppy and a cherry barb. To our knowledge, no quantitative data are available on the comparative *in vitro* I^{131} uptake by fish tissues. In the present paper,data on the *in vitro* I^{131} uptake by different tissues of rainbow trout under different experimental conditions are presented.

Material and Methods

Fish Tissues.—Different tissues from one-year-old trout (*Salmo gairdnerii*), which were kept at 13° ± 0.5°C. under constant illumination in 26-gallon glass aquaria, were used in all experiments. Fish were anesthetized by immersion for 15 to 20 seconds in 0.12 per cent aqueous solution of MS-222 (composition: tricaine methanesulforate, Sandoz Chemical Works, N.Y.). A region of the lower jaw was dissected out and cut into small pieces. Some gills (gill arch plus filaments) were divided into upper (dorsal) and lower (ventral) portions, whereas others were separated into their component gill arches and gill filaments.

Pieces of lower jaw, gills and their various parts, stomach, caeca, intestine, kidney, heart, skeletal muscle, head kidney, spleen, liver and brain tissues were dissected out and chilled over ice. Slices of tissue weighing from 30 to 80 mg. were prepared and kept in diluted Nandi and Bern's[10] medium until the radioiodine was added and the incubation period begun.

Medium.—Nandi and Bern's medium containing the following concentrations of solutes in millimoles per liter was prepared: NaCl, 200; KCl, 5.0; $CaCl_2$, 3.0; $MgCl_2$, 2.0; $NaHCO_3$, 1.0; NaH_2PO_4, 10.0; glucose, 6.0; water, 1,000 ml. This solution was made isosmotic to the blood of rainbow trout by dilution with distilled water, the pH adjusted to 7.3 with phosphate buffer. and stored in the refrigerator at 7°C.

Radioiodine.—I^{131} was obtained from Oak Ridge National Laboratory and was diluted with glass distilled water to a concentration of 0.3μc/0.1 ml., the quantity added to each flask along with 2 ml. of the medium noted above.

Antithyroid Agents.—Aqueous solutions of antithyroid agents were prepared and aliquots (0.1 ml.) added to 2 ml. of the medium to give the following concentrations: thiouracil, 0.02 mg./ml.; KSCN, 0.2 mg./ml.

Boiling of Tissues.—Tissue slices were kept in boiling water for one minute.

Counting Equipment.—A scintillation well-type counter was used to determine the activity in both the tissue and the incubation medium.

Tissue slices from different organs of rainbow trout were added to each flask containing 2 ml. of the medium and then various substances were added, depending upon the experiment. To each flask I^{131} was added (see above) and the flasks

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were incubated, with agitation, in a water bath at 13°C. for 2 hours because the maximum I^{131} uptake was observed at the end of two hours' incubation. The flasks were then taken from the water bath, the tissue removed from each flask, blotted, rinsed with glass-distilled water to remove I^{131} sticking to its surfaces, again blotted and then placed in a previously weighed glass counting vial. The vials with the tissues were then weighed and the tissue weights recorded. One-tenth ml. of the incubation medium from each flask was pipetted into a counting vial and the volume made up to 0.2 ml. with water. Each smaple was counted for at least one minute and the range was 1—3 minutes. The T/M value for each tissue sample was calculated as follows :

$$\text{T/M value} = \frac{\text{Counts/minute/100 mg.tissue}}{\text{Counts/minute/0.1 ml. medium}}$$

All tissue samples were run in duplicate and each T/M value represents the mean of two observations.

Results

I^{131} Uptake by Gills.—Table 1 shows the comparative I^{131} uptake by different parts of gills. The T/M values for gills show that the uptake of I^{131} was greater in the lower than in the upper portion (Fig. 1). The same relationship holds for uptake by upper and lower parts of the gill arches (Table 1). The T/M values for whole gills were slightly above the values for filaments alone. The T/M values for boiled gill parts ranged from 0.475 to 0.683. Addition of thiouracil (0.02 mg./ml.) to the medium did not effect I^{131} uptake by the lower part of gill arches; however, KSCN (0.2 mg./ml.) caused a decrease in the T/M value (Table 1).

I^{131} Uptake by Lower Jaw Tissue.—The radioiodine uptake by portions of the lower jaw indicated that the posterior region contains comparatively more thyroidal follicles than anterior portion. The T/M values for anterior and posterior parts of

TABLE 1.—*In Vitro* UPTAKE OF I^{131} BY DIFFERENT TISSUES OF RAINBOW TROUT, INCUBATED AT 13±0.5°C. FOR TWO HOURS UNDER DIFFERENT EXPERIMENTAL CONDITIONS. THE VALUES REPRESENT THE COUNTS PER MINUTE PER 100 MG. OF TISSUE DIVIDED BY THE COUNTS PER 0.1 ML. OF THE MEDIUM (T/M RATIO). EACH T/M RATIO IS THE MEAN OF AT LEAST TWO OBSERVATIONS.

Tissue	T/M Values							
	Control		Boiled[1]		TH-treated[2]		KSCN-treated[3]	
	$\bar{X}$	Range	$\bar{X}$	Range	$\bar{X}$	Range	$\bar{X}$	Range
Cardiac muscle ...	.645	.614-.676	.623	.614-.632	.649	.619-.679	.626	.612-.640
Skeletal muscle ...	.573	.521-.625	.663	.645-.681	.601	.582-.620	.614	.594-.634
Intestine ...	.672	.561-.783	—	—	.682	.620-.660	.638	.617-.659
Kidney ...	.685	.671-.699	—	—	.678	.704-.667	.637	.586-.688
Head kidney ...	.696	.651-.741	—	—	.704	.683-.725	.678	.637-.719
Caeca	.746	.718-.774	—	—	.711	.674-.748	.747	.726-.768
Brain	.814	.804-.824	—	—	.821	.790-.852	.803	.801-.805
Spleen	.878	.817-.939	—	—	.846	.814-.878	.865	.783-.987
Liver	.892	.791-.993	.784	.743-.825	.863	.851-.875	.817	.802-.829
Stomach ...	.956	.938-.974	.882	.861-.903	.963	.942-.984	.946	.915-.977
Gills	.968	.914-1.002	.617	.605-.629	.957	.916-.998	.743	.714-.772
Gill filament ...	.836	.816-.856	.584	.475-.673	.874	.813-.935	.614	.601-.627
Gill arch ...	1.167	1.068-1.266	.636	.618-.654	1.184	1.062-1.306	.683	.642-.724
Gill arch (upper) ...	.879	.819-.939	.645	.604-.686	.914	.902-.926	.672	.632-.712
Gill arch (lower) ...	1.132	1.101-1.163	.683	.672-.694	1.107	1.016-1.198	.714	.682-.746

1. Tissues kept in boiling water for one minute 2. Thiouracil 0.02 mg./ml. 3. KSCN 0.2 mg./ml.

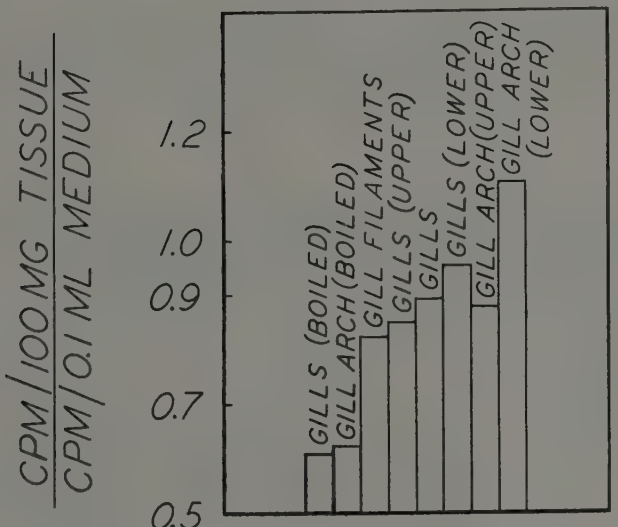

Fig. 1.—*In vitro* I131 uptake by different parts of gills of rainbow trout, incubated at 13°C. for two hours. Each T/M value represents the mean of two observations.

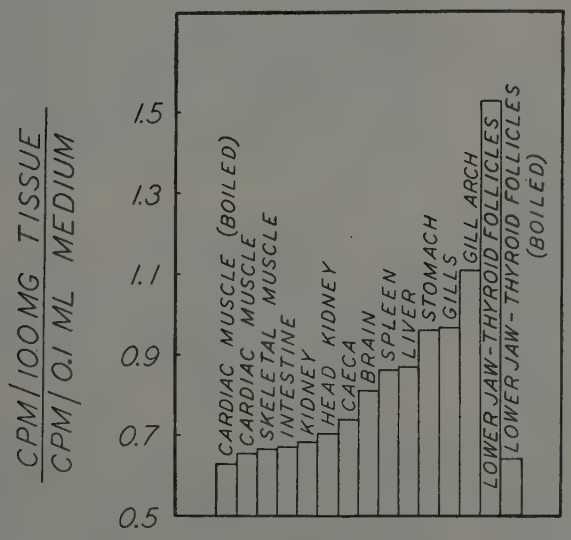

Fig 2.—*In vitro* I131 uptake by different tissues of rainbow trout. incubated at 13°C. for two hours. Each T/M value represents the mean of two observations.

the lower jaw were 1.143 and 1.784, respectively. The results presented graphically in Fig. 2 show that I131 uptake by the thyroid-containing lower jaw was higher than that of other tissues of rainbow trout. Since the lower jaw contains considerable tissue which does not actively take up radioiodine, the T/M values shown for this tissue do not represent true T/M values for thyroid follicles alone. Addition of thiouracil (0.02 mg./ml.) to the medium did not effect I131 uptake by lower jaw tissue. On the other hand, addition of KSCN caused a decrease in I131 uptake resulting in a T/M value close to that of boiled tissue (Table 1).

Uptake of Radioiodine by Organs of the Digestive System and Liver.—The T/M values for I131 uptake by tissues from the oesophagus, stomach, pyloric caeca, intestine and liver ranged from 0.443 to 0.974 (Figure 2). Addition of thiouracil and KSCN to the medium did not effect I131 uptake by these tissues (Table 1).

I131 Uptake by Other Tissues.—The T/M values for heart and skeletal muscles were 0.645 and 0.573 respectively. The values for the boiled tissues did not differ from those of the fresh ones. The T/M values for spleen and brain were some-

what higher than those for the kidney and head kidney. Addition of thiouracil and KSCN to the medium did not effect the I^{131} uptake of any of these tissues (Table 1).

Discussion

It is well established that thyroidal tissue in trout [5,11,12] consists of scattered follicles in the lower jaw region. The data presented here indicate, on the basis of I^{131} accumulation, that the posterior portion of the lower jaw contains relatively more thyroidal tissue than does the anterior portion. The lower portions of gills and gill arches consistently showed a greater uptake of I^{131} than did the more dorsally located portions. This, in all probability, is due to the presence of some thyroidal tissue in the former which was not removed during the dissecting procedure.

In vivo studies of the effect of goitrogens on fish have been summarized by Pickford and Atz.[3] Nearly all freshwater teleosts respond to antithyroid drugs with a marked hyperplasia of thyroidal tissue. The response may be rather slow and often it takes several days' exposure before any response is observable. Astwood[13] points out that *in vitro* addition of thiouracil to mammalian thyroid tissue prevents organic binding of iodine and that thiocyanates blocked the concentration of iodide. Data presented here for rainbow trout indicates that *in vitro* addition of thiouracil has no immediate effect on the uptake of I^{131} by lower jaw tissue (and presumably specifically on thyroid tissue) under the given conditions. These results should be considered in light of Hoar's[4] observation that the conversion of inorganic iodide to organically bound iodine in the thyroid of fish appears to be a rather slow process when compared to mammals and might be connected with the rate of oxidative processes which are independent of thyroxine levels. On the other hand, the blockage of I^{131} uptake by lower jaw tissue of trout with KSCN is similar to the effect this compound has on mammalian thyroidal tissue.

With the exception of lower jaw tissue, the T/M ratios for the various tissues studied indicate that relatively little iodide concentration takes place in these tissues. In this respect fish differ from mammals in which thyroidal, mammary gland, salivary gland and stomach tissues have been shown to concentrate iodide *in vitro*.[1,2] The fact that the various fish tissues showed T/M values higher than those for boiled tissues indicates that some uptake of iodide did occur. Although we have no proof, it may be that this small amount of uptake of radioiodine by the various tissues represents the activity of heterotopic thyroidal tissue. Since KSCN had no effect on the uptake, this process must differ from that occurring in the lower jaw area.

Summary

The data on I^{131} uptake by different tissues of rainbow trout indicate that only lower jaw tissue concentrated I^{131} to any significant degree. The T/M value (counts per minute per 100 mg. tissue divided by counts per minute per 0.1 ml. medium) was higher for tissue from the posterior part of the lower jaw than from the anterior part, indicating that the thyroid follicles are comparatively more numerous in the posterior part. I^{131} uptake was higher in the lower part of the gill than the upper part. This is probably due to the presence of some thyroid follicles in the tissue at the base of the gills at the point of attachment to the lower jaw. Addition of thiouracil to the medium did not affect I^{131} uptake by any of the tissues studied, whereas KSCN blocked I^{131} uptake by lower jaw tissue but had no effect on any other tissues. The T/M values for I^{131} uptake by different organs of the digestive system, kidney, head kidney, liver, spleen, heart, skeletal muscle and brain was less than one, indicating that these organs or tissues have a relatively poor ability to concentrate iodide *in vitro*.

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PRELIMINARY STUDY OF ANOMALOUS LAUE SPOTS OF ANTHRAQUINONE

A. M. CHAUDHURY AND A. K. BHUIYA*

Physics Department, University of Dacca, Dacca

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Various workers have investigated the diffuse radial streaks which appear along with the Laue photographs and various theories have been put forward to account for the results obtained. These, however, are not consistent and are sometimes contradictory.

The present authors have made a preliminary study of the anomalous Laue spots of anthraquinone and have observed that the position of the anomalous spots varies with the orientation of the crystals but the direction of the movement of these spots are not related in any way with the direction of the movement of the Laue spots. The data obtained also do not agree with the Faxen or Zachariasen's formula.

Introduction

The appearance of extra diffuse radial streaks in Laue photographs was first observed by Friedrich,[1] and at first Faxen[2] suggested that these streaks might be due to diffuse scattering of incident continuous X-ray by Debye heat waves produced in the crystal by thermal agitation. He later showed that when a monochromatic beam of X-rays is incident on a set of lattice planes there is a maximum in the intensity of the diffuse scattering which lies in the neighbourhood of the Laue spot. So, when white radiation is used, these diffuse spots with maxima would be drawn out as streaks.

The same problem was investigated by many other workers previously, who put forward different theories to account for the results obtained. The present work was undertaken in an attempt to achieve clarification of the apparent contradictions in the experimental result and interpretations of the previous investigators.

Experimental Procedure

(*a*) *Goniometry and Mounting of the Crystal.*—Anthraquinone $C_{14}H_8O_2$ which has been studied in the present case was crystallised from benzene. It belongs to the monoclinic system. Short needle-shaped crystals were obtained, the needle length being parallel to the b-axis. The important physical data are:—Anthraquinone $C_6H_4\langle^{CO}_{CO}\rangle C_6H_4$, Crystallises in holohedral class of the monoclinic system, a=15.85 Å, b=3.98Å, C=7.92 Å, β=102°7′, melting point 273°C., density 1.419. Space group, C^5_2 ($P2_1/a$). Two molecules per unit cell. Volume of the unit cell 487A°.[3] Total number of electrons per unit cell=F(000)=216. For the mounting of the crystal, a Czapski two-circle theodolite goniometer was used. A single and unstrained crystal was chosen, the angular measurements for which are given below in Table 1.

TABLE 1.—GONIOMETRIC MEASUREMENT.

Faces	Indices of the planes	Angle observed
a : c	(100) : (001)	77°19′
c : m	(001) : ($\bar{2}$01)	51°14′
m : a	($\bar{2}$01) : (100)	51°24′

It was then mounted on the goniometer head so that the 'a' face was normal to the X-ray beam. A small quantity of aluminium powder was dusted over the crystal for the measurement of the diameter of the camera and for comparing the intensity of the anomalous spots.

(*b*) *Description of the Camera and taking of Photographs.*—A cylindrical camera has been used chiefly for the following reasons: It extends the angular range of observation to a great extent, and also minimises the falling off of the intensity with the increase of the scattering angle. After first adjusting the crystal and fixing the goniometer head in the camera, with the X-ray beam passing normal to 'a', a number of photographs were taken with crystal at different orientations, the time of exposure being 7 to 8 hours (see photographs 1 and 2).

Indexing of Laue Spots

For indexing of the Laue spots the exact radius of the film was calculated by the formula $\psi=2x/D$,

*Now at Physical Research Division, Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi.

TABLE 2.—INDEXING OF THE LAUE SPOTS IN THE VICINITY OF THE ANOMALOUS SPOTS.

Angular co-ordinates of Laue spots		hλ	kλ	lλ	hkl
ψ in degrees	μ in degrees				
25°49′	0	—1.5818	0	3.539	$\bar{1}02$
54°8′	0	—6.5635	0	6.981	$\bar{4}04$
57°48′	23°27′	—8.104	1.504	6.887	$\bar{5}14$
43°54′	23°27′	—5.374	1.584	—4.314	$\bar{3}1\bar{3}$
28°2′	25°10′	—3.188	—1.692	3.636	$\bar{2}1\bar{2}$
17°24′	30°52′	—2.867	—2.042	2.297	$\bar{2}\bar{1}1$

TABLE 3.—DETERMINATION OF △ FOR THE ANOMALOUS SPOTS OF ANTHRAQUINONE

Indices of the planes giving rise to the anomalous spots	Angular co-ordinates of anomalous spots		$2\theta_i$	$2\theta_B$	$\triangle=\theta_i-\theta_B$
	ψ	μ			
$\bar{1}02$	25°49′	0	25°49′	25°20′	0°14′
,,	26°14′	0	26°14′	,,	0°27′
,,	27°12′	0	27°12′	,,	0°56′
,,	27°54′	0	27°54′	,,	1°17′
,,	29°24′	0	29°24′	,,	2°2′
$\bar{4}04$	54°8′	0	54°8′	53°47′	0°10′
,,	54°32′	0	54°32′	,,	0°22′
,,	54°42′	0	54°42′	,,	0°22′
,,	54°56′	0	54°56′	,,	1°4′
,,	57°14′	0	57°14′	,,	1°45′
$\bar{5}14$	57°48′	23°27′	60°46′	55°13′	2°46′
,,	58°41′	24°22′	61°45′	,,	3°16′
,,	58°57′	24°22′	61°57′	,,	3°22′
,,	60°3′	24°36′	63°	,,	3°53′
,,	62°32′	25°44′	65°27′	,,	5°7′
$\bar{3}1\bar{3}$	—43°54′	23°27′	48°38′	48°31′	0°3′
,,	—42°48′	23°27′	47°42′	,,	—0°10′
,,	—42°15′	23°41′	47°13′	,,	—0°39′
,,	—42°48′	21°21′	46°53′	,,	—0°49′
,,	—41°24′	22°45′	46°15′	,,	—1°8′
$\bar{2}1\bar{2}$	28°2′	—25°10′	36°58′	32°30′	2°14′
,,	26°55′	—24°2′	35°30′	,,	1°30′
,,	27°21′	—24°2′	35°49′	,,	1°39′
,,	28°43′	—27°4′	38°39′	,,	3°4′
,,	28°43′	—25°55′	37°56′	,,	2°43′
$\bar{2}\bar{1}1$	17°24′	—30°52′	35°2′	26°36′	4°13′
,,	16°34′	—23°46′	28°42′	,,	1°3′
,,	17°16′	—29°0′	23°22′	,,	3°23′
,,	17°49′	—32°34′	36°40′	,,	5°2′
,,	17°40′	—30°2′	34°25′	,,	3°54′

Photograph 1.

where 2x is the distance between the two corresponding lines, D is the diameter of the film and ψ is the angle of diffraction (in radians). Thus with the help of radius as determined above, the angular co-ordinates ψ and μ of the Laue spots were determined from the relation,

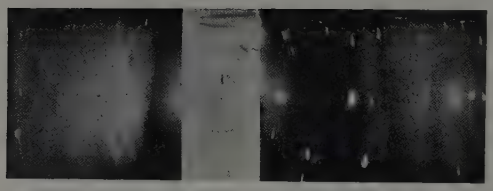
$$\psi = \frac{x}{R} ; \qquad \mu = \tan^{-1} \frac{e}{R}$$

where x is the horizontal distance of the film from a vertical line through the centre of the undeviated beam, e is the vertical distance from the equitorial layer lines and R is the radius of the film.

Here in the case of anthraquinone, the x-ray

TABLE 4.—DETERMINATION OF $\triangle$ FOR THE LAUE SPOTS OF ANTHRAQUINONE.

Indices of the planes giving rise to the Laue spots	Angular co-ordinates of Laue spots		$2\theta_i$	$2\theta_B$	$\triangle = \theta_i - \theta_B$
	ψ	μ			
$\bar{1}02$	24°34′	0	24°34′	25°20′	—0°23′
,,	24°26′	0	24°26′	,,	—0°27′
,,	24°10′	0	24°10′	,,	—0°25′
,,	24°26′	0	24°26′	,,	—0°22′
,,	24°43′	0	24°43′	,,	—0°18′
$\bar{4}04$	53°18′	0	53°18′	25°20′	—0°14′
,,	53°26′	0	53°26′	,,	—0°10′
,,	53°18′	0	53°18′	53°18′	—0°14′
,,	55°14′	0	5o°14′	,,	—0°43′
,,	55°30′	0	55°30′	,,	0°51′
$\bar{5}14$	58°50′	23°27′	61°40′	55°13′	3°13′
,,	57°59′	24°36′	61°11′	,,	2°59′
,,	57°59′	23°27′	60°54′	,,	2°50′
,,	59°20′	24°23′	60°19′	,,	3°33′
,,	60°44′	24°36′	63°36′	,,	4°11′
$\bar{3}1\bar{3}$	—42°48′	22°52′	47°28′	48°31′	—0°31′
,,	—40°51′	23°41′	46°9′	,,	—1°11′
,,	—41°34′	23°27′	46°40′	,,	—0°55′
,,	—43°26′	22°10′	47°43′	,,	—0°24′
,,	—42°32′	23°41′	47°34′	,,	—0°29′
$\bar{2}\bar{1}2$	28°2′	—25°24′	37°8′	32°30′	2°19′
,,	27°37′	—24°29′	36°16′	,,	1°53′
,,	27°37′	—24°15′	36°7′	,,	1°48′
,,	28°19′	—26°18′	37°54′	,,	2°42′
,,	28°19′	—25°17′	37°15′	,,	2°22′
$\bar{2}\bar{1}1$	15°11′	—26°51′	30°36′	30°36′	2°
,,	14°54′	—24°50′	28°43′	,,	1°6′
,,	14°54′	—24°50′	28°43′	,,	1°6′
,,	—	—	—	—	—
,,	15°11′	—24°57′	28°55′	,,	1°9′



Photograph 2.

beam was incident on the crystal along the a-axis while b-axis was vertical. If α_o, β_o γ_o and α, β, γ, are the direction cosines of the incident and diffracted beam respectively, then

$$\alpha_o=1, \quad \beta_o=0, \quad \gamma_o=\cos\delta.$$

Hence because the crystal belongs to the monoclinic system with the monoclinic angle, $\beta=102°$ 7′, therefore, for the diffracted beam,

$$\alpha=\cos\psi.\ \cos\mu, \quad \beta=\sin\mu$$

and $\gamma=\beta\cos\mu.\ \cos(\beta-\psi)$

TABLE 5.—COMPARISON OF $(2\theta_i+\psi)$ WITH $2\theta\beta$ FOR THE PLANES $\bar{1}02$, $\bar{4}04$, $\bar{5}14$, $\bar{3}1\bar{3}$, $\bar{2}\bar{1}2$, AND $\bar{2}\bar{1}1$ AS MEASURED BY OBSERVATION AND AS CALCULATED FROM FAXEN'S FORMULA.

Indices of the planes giving rise to anomalous spot	$\triangle=\theta_i-\theta_B$	$2\theta_B$	$(\theta_i+\varphi)$ observed	$(\theta_i+\varphi)$ as calculated from Faxen's formula	Difference Obs.-Cal.
$\bar{1}02$	0°14′	25°11′	25°11′	23°46′	1°25′
,,	0°27′	,,	25°20′	23°47′	1°33′
,,	0°56′	,,	25°41′	23°50′	1°51′
,,	1°17′	,,	26°10′	23°51′	2°19′
,,	2°2′	,,	27°7′	23°54′	3°13′
$\bar{4}04$	°010′	53°47′	53°43′	51°14′	2°29′
,,	0°22′	,,	53°59′	51°17′	2°42′
,,	0°22′	,,	54° ′	51°19′	2°41′
,,	1°4′	,,	55°35′	51°32′	4°3′
,,	1°45′	,,	56°24′	51°46′	4°38′
$\bar{4}14$	2°46′	55°13′	61°14′	57°35′	3°39′
,,	3°16′	,,	61°27′	57°47′	3°4′
,,	3°22′	,,	61°25′	57°49′	3°36′
,,	3°53′	,,	62°39′	58°8′	4°31′
,,	—	—	—	—	—
$\bar{3}1\bar{3}$	0°3′	48°31′	38°3′	46°55′	1°8′
,,	—0°10′	,,	46°56′	46°45′	0°10′
,,	—0°39′	,,	46°56′	46°41′	0°15′
,,	—0°49′	,,	47°17′	46°37′	0°40′
,,	—1°8′	,,	46°54′	46°20′	0°34′
$\bar{2}\bar{1}2$	2°14′	32°30′	37°3′	33°41′	3°22′
,,	1°30′	,,	35°53′	33°34′	2°19′
,,	1°39′	,,	35°53′	35°35′	0°22′
,,	3°4′	,,	38°16′	33°48′	4°30′
,,	2°43′	,,	37°35′	33°45′	3°50′
$\bar{2}\bar{1}1$	4°13′	26°36′	32°49′	27°43′	5°6′
,,	1°3′	,,	28°42′	27°32′	1°10′
,,	3°23′	,,	31°2′	27°37′	3°25′
,,	5°2′	,,	—	—	—
,,	3°55′	,,	31°39′	27°41′	3°58′

Thus from the Laue equations, we have,

$$\frac{a(\cos\psi\ \cos\mu - 1)}{b(\sin\mu)} = \frac{h}{k},$$

$$\frac{b(\sin\mu)}{c[\cos\mu\{\cos\ (\delta-\psi)\}-\cos\delta]} = \frac{K}{L}$$

Thus we can find h/k and k/l (axial length of the crystal being known). Laue spots of other photographs rotated to different orientations could be followed and indexed in the same manner. See photograph No. 2 where Miller indices of six spots calculated in the above described manner are shown.

Study of the Anomalous Spots

The study of the anomalous spots, the measurement of their exact positions and their comparison

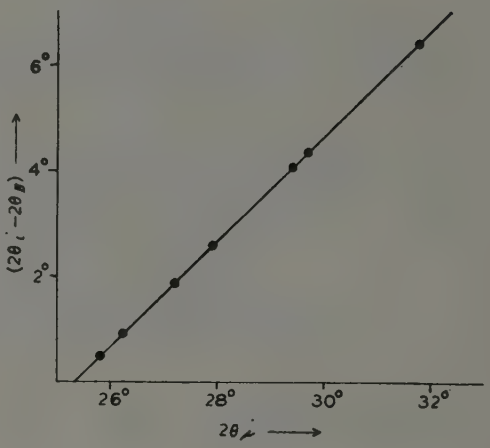
TABLE 6.—COMPARISON OF $(2\theta_i+\varphi)$ WITH $2\theta_B$ FOR THE PLANES $\bar{1}02$, $\bar{4}04$, $\bar{5}14$, $\bar{3}1\bar{3}$, $\bar{2}1\bar{2}$, AND $\bar{2}1\bar{1}$ AS MEASURED BY OBSERVATION AND AS CALCULATED FROM ZACHARIASEN'S FORMULA.

Indices of the planes giving rise to anomalous spots	$\triangle=\theta_i-\theta_B$	$2\theta_B$	$(\theta_i+\varphi)$ observed	$(\theta_i+\varphi)$ as calculated from Zachariasen's formula	Difference Obs.-Cal.
$\bar{1}02$	0°14′	25°20′	25°11′	25°21′	0°10′
,,	0°27′	,,	25°20′	25°22′	—0°2′
,,	0°56′	,,	25°41′	25°25′	0°16′
,,	1°17′	,,	26°10′	25°27′	0°43′
,,	2°2′	,,	27°7′	25°31′	1°36′
$\bar{4}04$	0°10′	53°47′	53°43′	53°51′	—0°8′
,,	0°22′	,,	53°59′	53°56′	0°3′
,,	0°22′	,,	54°′	53°56′	0°4′
,,	1°4′	,,	55°35′	54°12′	1°23′
,,	1°45′	,,	56°24′	54°29′	1°55′
$\bar{5}14$	2°46′	55°13′	61°14′	56°23′	4°51′
,,	3°16′	,,	61°27′	56°31′	4°56′
,,	3°22′	,,	61°25′	56°33′	4°52′
,,	3°53′	,,	62°39′	56°50′	5°49′
,,	—	—	—	—	—
$\bar{3}1\bar{3}$	0°3′	48°31′	48°3′	48°32′	0°29′
,,	—0°10′	,,	46°55′	48°28′	—1°13′
,,	—0°39′	,,	46°56′	48°18′	—1°12′
,,	—0°49′	,,	47°17′	48°15′	—0°58′
,,	—1°8′	,,	46°54′	48°9′	—1°15′
$\bar{2}1\bar{2}$	2°14′	2°330′	37°3′	32°50′	4°13′
,,	1°30′	,,	35°53′	32°44′	3°9′
,,	1°39′	,,	35°57′	32°45′	3°12′
,,	—	,,	—	—	—
,,	2°43′	,,	37°35′	32°55′	4°40′
$\bar{2}1\bar{1}$	1°3′	26°36′	28°42′	26°42′	2°
,,	3°23′	,,	31°2′	26°57′	4°5′
,,	5°2′	,,	—	—	—
,,	3°54′	,,	31°39′	27°′	4°39′
,,	4°16′	,,	32°22′	27°3′	5°9′

with those of the corresponding Laue spots is first required. For the anomalous spots in the central layer, the distance of the spot was measured from the nearest aluminium line so that the angular separation from the aluminium line was easily calculated from the actual radius of the camera. The scattering angle of aluminium being known, the angle between the direct ray and the direction of the anomalous spot was thus accurately known. For the spots above or below the central layer, the co-ordinates ψ_a and μ_a were evaluated and $2\theta_a$ the total scattering angle of the anomalous spot was obtained from the relation $2\theta_a = \cos\psi_a . \cos\mu_a$. In this way the spots corresponding to $(\bar{1}02)$, $(\bar{4}04)$, $(5\bar{1}4)$, $(\bar{3}1\bar{3})$, $(2\bar{1}2)$ and $(2\bar{1}1)$ have been studied in different photographs. It was observed from these measurements that the position of the anomalous spot, varied with the orientation of the crystal, i.e. the Laue spots. It was also observed that the direction of movements of the anomalous spots were not related in any way with the direction in which the Laue spots moved.

Table 2 gives some details of the indexing of the Laue spots while Table 3 gives the angular co-ordinates of the anomalous spots associated with the $\bar{1}02$, $\bar{4}04$, $5\bar{1}4$, $\bar{3}1\bar{3}$, $2\bar{1}2$ and $2\bar{1}1$ reflection together with the calculated value of $\triangle$, for various settings of the crystal.

The variations of $(2\theta_i - 2\theta_a)$ with $2\theta_i$ were studied to identify the association of anomalous spot with a given Laue spot. The values of $(2\theta_i - 2\theta_a)$ were plotted against the values of $2\theta_i$ in a graph. The most interesting and important result was that observed from this graph was that all the curves were straight lines. This shows that the variation of $(2\theta_i - 2\theta_a)$ with $2\theta_i$ are regular and directly proportional to each other. This was not predicted in any of the existing theories. Moreover, this straight line intersects the $2\theta_i$ axis at a point whose value is equal to $2\theta_b$ for that spot for Cu $K\alpha$ well within the limits of experimental error. It means that when the difference is zero, the anomalous spot coincides with the Laue spot and the scattering angle for the combined spot is equal to $2\theta_B$ (see Graph 1).



Graph 1.

Discussion

In course of the present studies the following facts have been observed.

The position of the anomalous spot varies with the orientation of the crystal. However, Table 3 shows that the directions of movement of the anomalous spots are not related in any way with the direction in which the Laue spots move. A comparison with theory is made in Table 5 and Table 6 below, but it is seen that the agreement with either Faxen's or Zachariasen's formula is poor. The differences between the observed and the calculated values of $(\theta_i + \varphi)$ are considerably greater than the limits of experimental error in this experiment for the larger values of $\triangle$, i.e. greater than 2°.

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STUDIES IN THE PROPERTIES OF HEAT INSULATING BUILDING MATERIALS

Part III.—Analysis of the Anomalous Thermal Conductivity of Blocks of Cement and Rice Husk Ash

MAZHAR M. QURASHI AND A.K.M. AHSANULLAH

Physical Research Division, Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi

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The new $K^{\frac{1}{3}}$ formula derived in an earlier paper for the thermal conductivity of simple binary mixtures of two components has now been applied to the case of concretes of cement and rice husk ash. A proper allowance for the anomalous contraction is necessary, and a theoretical evaluation of this rather large correction is carried out. When this correction is made, the calculated values are in approximate agreement with the observed data. The outstanding error of $17 \pm 17\%$ is correlated with the formulation of new products by chemical reaction of the activated silica (of the ash) with the cement, but could be partly due to a suspected anomaly in the properties of the local cement itself.

1. Theoretical Conductivity without Allowance for Contraction

In Part II of this series,[1,2] an analysis was presented for the thermal conductivity of a simple mixture of x_a and x_b parts by volume of two components with thermal conductivities K_a and K_b respectively, and the following experession was obtained:[2]

$$K_{mix}^{\frac{1}{3}} = \frac{x_a}{x_a + x_b} K_a^{\frac{1}{3}} + \frac{x_b}{x_a + x_b} K_b^{\frac{1}{3}} \qquad (1)$$

Experimental evidence for the correctness of this formula was obtained both from the standard data on cellular concretes and from our measured values for rice husk ash, assuming that both these materials are binary mixtures of air with the solid component.

Formula (1) when applied to binary mixtures of cement with rice husk ash gives the values of conductivity shown in the third row of Table 1 for various compositions by volume. A comparison of these calculated values with those determined experimentally for concretes of rice husk ash[1] is shown in Fig. 1(a). It is at once apparent that the experimental values are all higher than those calculated in Table 1, and that the ratio of the two rises to a maximum of nearly $2\frac{1}{2}$ at about 80% of ash by volume. Now, if we ignore the anomalous maximum and minimum in the experimental curve and draw a monotonically decreasing graph, we obtain the broken line curve Fig. 1(a). The relation between this curve and the calculated one is very similar to that between the measured densities and those expected on the basis of simple mixing without contraction (Fig. 1 (b)), thus suggesting that the major part of the discrepancy observed in Fig. 1(a) can be explained by the large contraction in volume.

TABLE 1.—VALUES OF K_{ideal} OBTAINED ON THE BASIS OF AN IDEAL BINARY MIXTURE.

Vol % ash:	0	20	40	60	80	100
$10 \times K_{ideal}^{\frac{1}{3}}$:	0.87_1	0.80_2	0.73_3	0.66_3	0.59_4	0.52_5
$10^3 \times K_{ideal}$:	0.66_0	0.51_5	0.39_3	0.29_1	0.21_0	0.14_5

2. Analysis of the Volume Contraction of the Rice Husk Ash Cement Blocks

If the apparent densities of the ash and the cement are ρ_{ash} and ρ_c, respectively, then the density of a simple mixture of x_{ash} parts by volume

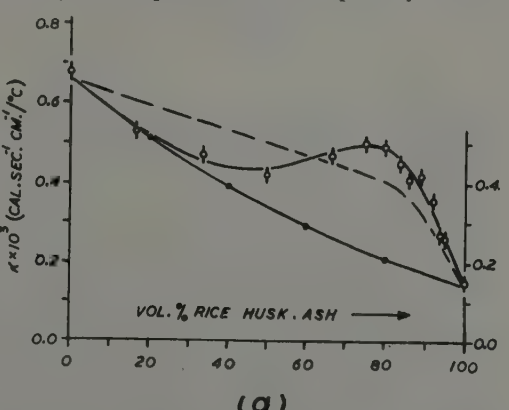
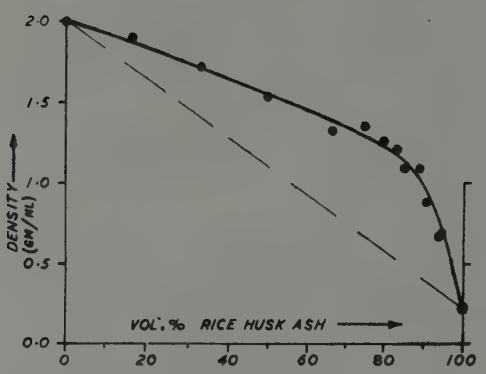


Fig. 1(a).—Comparison of experimentally measured thermal conductivities for concretes of rice husk ash (hollow circles) with K_{ideal} (solid circles) i.e. the values calculated on the basis of an ideal binary mixture. The broken line indicates the averaged monotonically decreasing component of the experimentally observed variation.



(b)

Fig. 1 (b).—The solid circles and full line show the experimental variation of the density of concretes of rice husk ash, while the broken line shows the variation for simple mixing without contraction.

of ash and ($1-x_{ash}$) parts of cement should be

$$\rho_{ideal} = \left\{ x_{ash}\,\rho_{ash} + (1-x_{ash})\,\rho_c \right\} \Big/ \left\{ x_{ash} + (1-x_{ash}) \right\} = \rho_c + (\rho_{ash} - \rho_c)\,x_{ash}, \qquad 2(a)$$

which corresponds to the broken line of Fig. 1(b). In order to study the contraction taking place on mixing various volumes of cement and rice husk ash, it is advantageous to calculate the quantity ρ_{ideal}/ρ_{expt} because the fractional volume contraction, $\triangle_f$, is given by

$$\triangle_f = (\text{Ideal Volume} - \text{Expt. Volume})/\text{Ideal Volume} = 1 - \rho_{ideal}/\rho_{expt} \qquad 2(b)$$

The values of ρ_{ideal}/ρ_{expt} and $\triangle_f$ as calculated from the experimental curve of Fig. 1(b) are tabulated in Table 2 and are shown graphically in Fig.2. This graph too shows an anomaly in that the contraction $\triangle_f$ is at first equal to $0.55 \times x_{ash}$, but (soon) increases faster than the volume of ash added. If the contraction is assumed to be purely mechanical, i.e. due to the cement entering the pores in the ash particles (Fig. 3), then the variation of $\triangle_f$ would be essentially as shown in Fig. 2 by either the dotted or the broken lines, whose slope is constant at first until nearly all the pores are filled up and then decreases continuously with increasing ash content. The broken line corresponds to filling up of all the vacant space available, which is 90% of the volume of the ash (whose apparent bulk density is one-tenth of that of silica), while the dotted curve corresponds to filling up of about 50% of the available space, which is comparable

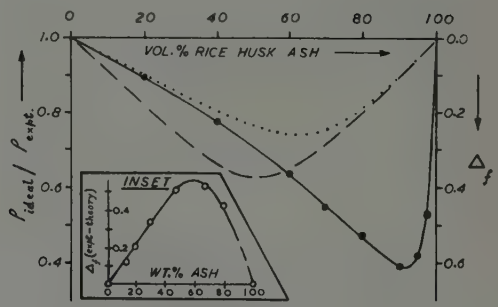


Fig. 2.—Solid circles and full line show the observed values of ρ_{ideal}/ρ_{expt} and the fractional contraction on mixing, Δf. The other lines show the expected contraction for filling up of (i) all the available space in the pores of the ash (broken line) and (ii) about 50% of the available space (dotted line).

Inset shows the excess of the experimental over the expected contraction, plotted against weight percent ash in the concrete.

Fig. 3.—A photomicrograph of a flake of the ash, showing the cellular structure (×100 approximately).

with the volume of the open region of the silical lattice-work of the ash seen magnified in Fig. 3.

In either case, the curve for a purely mechanical contraction should be essentially of a parabolic character with a maximum value of about 0.3 for Δ_f at 50% to 60% ash by volume. The experimental curve is comparable with the theory only for less than 60% ash, and the very prominent bump in the region of 80 to 100% ash must therefore be attributed largely to a contraction caused by a chemical reaction between the silica and the cement. This excess contraction can be estimated from the figure, and is seen to reach a maximum of over 0.5 at 93% ash by volume, i.e. 60% by weight

(*cf.* inset to Fig. 2). Such a reaction in the cold is to be expected because the silica of the ash becomes activated in the course of burning the rice husk (where temperatures of the order of 700°C. are usual), and indeed use has previously been made of this type of reaction in the manufacture of 'Pozzolana' cements[3] from burnt clays, bricks, etc.

In order to examine the influence of this reaction on the thermal conductivity, we must at first separate out the effect produced through the purely mechanical contraction on the thermal conductivity of the composite concrete mixture.

3. Effect of Mechanical Contraction on Thermal Conductivity

If K_{ideal} is the thermal conductivity of a particular mixture as calculated in Table 1, ρ_{ideal} is the density calculated on the basis of no contraction, and ρ_{expt} is the actual density, then one might expect the experimental value of the conductivity to be increased to

$$K_{calc} = K_{ideal} \times \frac{\rho_{expt}}{\rho_{ideal}}$$

the line of argument being that there is now $(\rho_{expt}/\rho_{ideal})$ times as much conducting material in a unit cube as before. This naive reasoning, ignores the varying influence of the large volume of air entrained inside the ash by virtue of its cellular structure. Since the measurements were made on samples oven-dried at about 110°C., of which water content was found to be between 4 and 10%, we may in the first instance, ignore its presence and consider the blocks as a three-component system comprising cement, silica, and air in varying proportions, x_c, x_s and x_a by volume. If the (effective) conductivities of these three materials are K_c, K_s and K_a, respectively, then from equation(1), the conductivity of the ash will be

$$K_{ash}^{\frac{1}{3}} = \frac{x_s}{x_s+x_a} K_s^{\frac{1}{3}} + \frac{x_a}{x_s+x_a} K_a^{\frac{1}{3}},$$

and thence the conductivity of the composite mixture will be

$$K_{ideal}^{\frac{1}{3}} = \frac{x_c}{x_c+(x_s+x_a)} K_c^{\frac{1}{3}} + \frac{x_s+x_a}{x_c+(x_s+x_a)} K_{ash}^{\frac{1}{3}}$$

$$= \left\{ x_c K_c^{\frac{1}{3}} + (x_s+x_a) \left(\frac{x_s}{x_s+x_a} K_s^{\frac{1}{3}} + \frac{x_a}{x_s+x_a} K_a^{\frac{1}{3}} \right) \right\} \div (x_c+x_s+x_a)$$

$$= \frac{x_c}{x_c+x_s+x_a} K_c^{\frac{1}{3}} + \frac{x_s}{x_c+x_s+x_a} K_s^{\frac{1}{3}} + \frac{x_a}{x_c+x_s+x_a} K_a^{\frac{1}{3}} \qquad 3\,(a)$$

$$= x_c K_c^{\frac{1}{3}} + x_s K_s^{\frac{1}{3}} + x_a K_a^{\frac{1}{3}} \qquad 3\,(b)$$

if $x_c + x_s + x_a = 1$

Now suppose that a contraction occurs on forming the mixture; this means that a fraction, say α, of the air is removed. We must therefore replace x_a by $(1-\alpha)\, x_a$, and $(x_c + x_s + x_a\,(1-\alpha))$ will be equal to $(1-\alpha x_a)$. So that the conductivity K_{calc} will be given by

$$K_{calc}^{\frac{1}{3}} = \frac{x_c}{1-\alpha x_a} K_c^{\frac{1}{3}} + \frac{x_s}{1-\alpha x_a} K_s^{\frac{1}{3}} + \frac{(1-\alpha)\, x_a}{1-\alpha x_a} K_a^{\frac{1}{3}}$$

$$= \left\{ (x_c K_c^{\frac{1}{3}} + x_s K_s^{\frac{1}{3}} + x_a K_a^{\frac{1}{3}}) - \alpha x_a K_a^{\frac{1}{3}} \right\} \div (1-\alpha x_a)$$

$$= (K_{ideal}^{\frac{1}{3}} - \alpha x_a K_a^{\frac{1}{3}}) / (1-\alpha x_a) \qquad 4\,(a)$$

Thus

$$(K_{calc}^{\frac{1}{3}} / K_{ideal}^{\frac{1}{3}}) = \left\{ 1-\alpha x_a \left(K_a^{\frac{1}{3}} / K_{ideal}^{\frac{1}{3}} \right) \right\} / (1-\alpha x_a)$$

$$= 1 + \left\{ 1 - (K_a / K_{ideal})^{\frac{1}{3}} \right\} \times \frac{\alpha x_a}{1-\alpha x_a} \qquad 4\,(b)$$

TABLE 2.—THE FRACTIONAL CONTRACTION, $\triangle f$, OBSERVED IN CONCRETES OF RICE HUSK ASH.

$100\, x_a$ = Vol % of ash :	0	20	40	60	70	80	90	95	97.5	100
$(\rho_{idea}/\rho_{expt})$	1.000	0.897	0.777	0.633	0.547	0.470	0.390	0.420	0.52	1.000
$\triangle f$	0.000	0.103	0.223	0.367	0.453	0.530	0.610	0.580	0.48	0.000
$\triangle f / x_a$	—	0.52	0.56	0.61	0.65	0.66	0.68	0.61	0.50	0.00

Now αx_a is merely the fractional contraction, measured as a fraction of the ideal volume for simple mixing. We therefore get.

$$\alpha x_a = \frac{\text{Ideal volume—Experimental volume}}{\text{Ideal volume}}$$

$$= \Delta_f$$
$$= 1 - (\rho_{ideal}/\rho_{expt}),$$

whence

$$\frac{\alpha x_a}{1-\alpha x_a} = (\rho_{expt}/\rho_{ideal})-1,$$

so that equation (4) becomes,

$$\left\{\frac{K_{calc}}{K_{ideal}}\right\}^{\frac{1}{3}} = 1+\left\{\frac{\rho_{expt}}{\rho_{ideal}}-1\right\}\left\{1-\left\{\frac{K_a}{K_{ideal}}\right\}^{\frac{1}{3}}\right\} \quad (5)$$

It is interesting to note here that $(K_a/K_{ideal})^{\frac{1}{3}}$ is equal to 0.6 ± 0.1 for blocks with K_{ideal} between 0.5×10^{-3} and 0.17×10^{-3} cal. cm^{-1} sec^{-1}/°C., and therefore we also get the approximation

$$\frac{K_{calc}}{K_{ideal}} \simeq \left\{1+\left\{\frac{\rho_{expt}}{\rho_{ideal}}-1\right\}(1-0{\cdot}6)\right\}^3$$

$$= \left\{0{\cdot}4\ \rho_{expt}/\rho_{ideal}+0{\cdot}6\right\}^3$$

$$\simeq \left\{\rho_{expt}/\rho_{ideal}\right\}^{1{\cdot}5} \quad \text{for } 1 < \rho_{expt}/\rho_{ideal} < 2.5,$$

which result is to be compared with the elementary notion mentioned previously that K_{calc}/K_{ideal} should be proportional to ρ_{expt}/ρ_{ideal}.

Formula (5) can be used to make an accurate allowance for the mechanical effect of the contraction in the case of concretes of rice husk ash, taking $K_a=0.06_0\times10^{-3}$ cal cm^{-1} sec^{-1}/°C. Table 3 gives the values of ρ_{expt}/ρ_{ideal} obtained from Fig. 1, of K_{ideal} (i.e. without contraction) and of K_{calc} i.e. with allowance for contraction, together with the ratio K_{expt}/K_{calc}, while Fig. 4 shows a comparison of K_{ideal} and K_{calc} with the experimentally determined values, K_{expt}.

4. Discussion of the Residual Effect

It is seen that, whereas the values of K_{ideal} are considerably lower than the experimental values (K_{expt}), the introduction of the effect of contraction brings K_{calc} into approximate agreement with experiment. However, the error is now in the opposite direction, K_{expt} being consistently lower than K_{calc}, with a mean value of about 0.83 for the ratio K_{expt}/K_{calc} which is a minimum in the region of 96% by volume of rice husk ash (about 70% by weight). This discrepancy may well be a result of some departures from the foregoing theory, or more probably of anomalous behaviour of the local cement, for which there is some evidence from other sources. Moreover, it generally follows the variation of Δ_f (expt—theory), and can therefore also be connected with the change in thermal conductivity brought about by the 'Pozzolana' type of reaction. If we take the abnormal fractional contraction observed in Fig. 2, i.e. Δ_f (expt.—theory) as being equal to β times the quantity of this new compound with thermal conductivity K_p, then to a first approximation, the ratio K_{expt}/K_{calc} in Table 3 should be given by

TABLE 3.—COMPARISON WITH EXPERIMENT OF THE VARIOUS THEORETICALLY CALCULATED VALUES OF K WITH AND WITHOUT ALLOWANCE FOR THE AIR IN THE PORES; $K_{air}=0.060\times10^{-3}$ cal. cm^{-1} sec^{-1}°C.

Vol. % ash	0	20	40	60	70	80	90	95	97.5	100
ρ_{expt}/ρ_{ideal}	1.000	1.114	1.287	1.580	1.828	2.126	2.563	2.38	1.83	1.000
$K_{ideal}\times10^3$ from eq. (1) ..	0.660	0.515	0.393	0.291	0.249	0.210	0.175	0.159	0.152	0.145
$(K_a/K_{ideal})^{\frac{1}{3}}$	0.450	0.488	0.535	0.592	0.622	0.658	0.700	0.723	0.734	0.746
$K_{calc}\times10^3$ .. .. from eq. (5)	0.660	0.610	0.570	0.550	0.564	0.555	0.555	0.420	0.276	0.145
$K_{expt}\times10^3$ (from graph) ..	0.66	0.525	0.44	0.46	0.485	0.485	0.37	0.26	0.20	0.145
K_{expt}/K_{calc}	1.00	0.86	0.77	0.84	0.86	0.87	0.67	0.62	0.72	1.00
Wt. % ash	0	2.5	6.2	13	19	29	47	66	80	100

TABLE 4.—COMPARISON OF HALF THE RESIDUAL ERROR $1/2\ (1-K_{expt}/K_{calc})$ WITH THE ANOMALOUS CONTRACTION Δ_f (EXPT—THEORY)

Weight % ash	10	20	30	40	50	60	70	80	90
Δ_f (expt-theory)	.12	.22	.33	.44	.54	.56	.52	.40	.22
$1/2\ (1-K_{expt}/K_{calc})$	.10	.07	.08	.12	.17	.18	.18	.14	.08
$0.31 \times \Delta_f$ (expt—theory)	.04	.07	.10	.13	.17	.17	.16	.12	.07

$$1-(K_{expt}\ /\ K_{calc}) \simeq (1-K_p\ /K_c\ )\times\beta x \qquad \Delta_f \text{ (expt.—theory)} \qquad (6)$$

If now we proceed on the likely assumption that only one-half of the discrepancy is due to this cause, then it should be possible to make a rough estimate of the quantity $[(1-K_p/K_c)\times\beta)]$ from a comparison of the two quantities $1/2\ (1-K_{expt}/K_{calc})$ and Δ_f (expt—theory). This comparison is made in Table 4, and a value of about 0.31 ± 0.03 can be deduced for $(1-K_pK_c)\times\beta$, cf. the last row of Table 4. From the graphs of Fig. 2, it is clear that β is considerably greater than 0.56, and since it is constrained to be less than 1.0, a fair estimate is 0.85 ± 0.1. It follows that i.e. the thermal conductivity of the new product of reaction with

$$1-K^p/K_c \simeq \frac{0.31\pm0.02}{0.85\pm0.1} = 0.36\pm0.06$$

whence

$$K_p/K_c \simeq 0.64\pm0.06,$$

the activated silica is about 2/3 that of ordinary cement (of zero porosity). The conductivity of the concretes calculated on this is shown in Fig. 4 by the chain-dotted line. Although this estimate may be vitiated very considerably by any major departures of the actual thermal behaviour from the theory outlined in the previous section, we may nevertheless conclude that the above theory taken together with the 'Pozzolana' type of reaction does account (to within about 15% as indicated by Table 4 and Fig. 4) for the anomalous

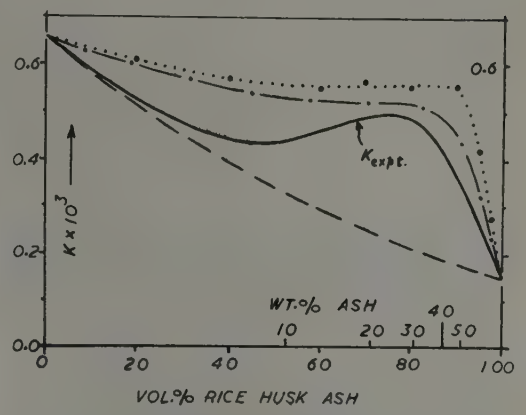


Fig. 4.—Graphs showing the measured thermal conduct i-vity K_{expt} of concretes of rice husk ash (full line) compared with (i) K_{ideal} (broken line) worked out on the basis of an ideal binary mixture, and (ii) K_{calc} (dotted line) calculated with allowance for contraction according to equation (5). The chain dotted curve, further includes an estimated correction for the effects of chemical reaction, and is seen to follow the general trend of the experimental curve.

behaviour of the thermal conductivity of concretes of rice husk ash. The suspected anomaly in the conductivity and other properties of cement at various porosities is being studied further in collaboration with the Building Materials Research Division, and is expected to remove the outstanding 15% discrepancy in the results on concretes of rice husk ash.

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GLUTAMIC ACID

Part II.—Glutamic Acid from Oil Cakes

MUHAMMAD ASHRAF* AND KARIMULLAH†
Institute of Chemistry, University of the Panjab, Lahore

(Received August 18, 1960)

There is an abundance of various oil cakes in Pakistan. The authors have carried out investigation on different oil cakes with respect to their glutamic acid content. It has been found that glutamic acid, glutamic acid hydrochloride and monosodium glutamate can be produced with maximum yield from rapeseed, cottonseed and linseed oil cakes. The technique of the production is quite easy and makes use of chemicals mostly available in Pakistan.

In Part I[1] of the present series of papers on the production of glutamic acid in Pakistan, various sources from which the acid can be manufactured, were enumerated. In the previous communication it was mentioned that oil cakes constitute an easily accessible and abundant source for the production of glutamic acid. The production of glutamic acid alone from food-grains would not be possible economically in view of the current food shortage in the country. If, however, the acid is produced as a by-product of the starch industry in Pakistan, the utilization of food grains for its production would not only be justifiable but desirable also. The earlier paper emphasised this point particularly.

However, various oil cakes are available in large quantities in Pakistan. At present they find little use as a human food. They are mainly consumed as animal feed and are also sometimes used even as fertilizers. An idea as to the quantity of the various oil cakes that are available in the country can be gained from Table 1. The data in the table were compiled from the figures for the production of various oil seeds for 1958 in both East and West Pakistan.[2]

The abundance of the various oil cakes in the country is thus quite clear. Their effective utilisation in itself calls for investigations. The proteins of these oil cakes can be recovered and used in fortification of protein-deficit diet. These proteins can also be employed for the production of several essential amino acids. Glutamic acid is one such essential acid.

In view of the value of glutamic acid both as an essential amino acid as well as a food adjunct, a study of its production from oil cakes in Pakistan was thus considered most desirable. Investigations were carried out on different oil cakes with respect to their glutamic acid contents. At the same time the production of the acid by cheap and economically workable methods was also studied. These studies are presented in the present paper.

TABLE 1.—PRODUCTION FIGURES OF IMPORTANT OIL SEED CAKES OF PAKISTAN (1958).

Oil cake	W. Pakistan	E. Pakistan	Total
Rape & mustard	123581 tons	50685 tons	174266 tons
Cotton	432915 ,,	3822 ,,	436737 ,,
Linseed	1299 ,,	7148 ,,	8447 ,,
Sesamum	2340 ,,	12636 ,,	14976 ,,
Castor	1656 mds.	20460 mds	22116 mds.
Ground-nut	Almost entire supplies are used as nuts.		

Materials

Oil Cakes.—Data in Table 2 have been given in order to compare the glutamic acid content of various oil cakes. It is observed that cottonseed

TABLE 2.—GLUTAMIC ACID CONTENT OF SOME OIL CAKES.[3]

Oil cake	Glutamic acid %
Groundnut	7.8
Cottonseed	7.5
Hempseed	6.4
Sunflower	5.2
Castor	5.6
Cocoanut	5.0
Linseed	3.8

* Now at the Department of Pharmacy, University of the Panjab, Lahore.

† At West Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Lahore.

cake has almost the same content of glutamic acid as soya-bean meal. But the percentage of glutamic acid available from mustard cake was found, in our investigations, to be the highest. As both rapeseed and cottonseed oil cakes constitute by far the largest quantity of oil cakes available in the country, these can go a long way to meet the requirements of glutamic acid production in Pakistan. Soya-bean meal is employed for similar purposes in other countries. Three main oil cakes used in these investigations were procured locally from the market.

Methods

The methods for the production of glutamic acid hydrochloride, glutamic acid and mono sodium glutamate were developed after a good deal of experimental work to give the maximum yield of these products. These methods are indicated as follows.

(*i*) *Glutamic Acid Hydrochloride.*—One hundred g. oil cake, 300 ml. hydrochloric acid (sp. gr. 1.14), and 1 g. tin were refluxed at 107-110°C. in one litre round-bottom flask for 12 hours. Afterwards the contents of the flask were allowed to cool to room temperature and filtered. The flask was washed with 15 ml. of concentrated hydrochloric acid, which was further used for washing the residue on the filter. The filtrate was boiled with 20 g. of activated animal charcoal for 15 minutes. The mixture was cooled to about 60°C. and then filtered. The filtrate obtained was again treated with 10 g. of the activated animal charcoal as before. The mixture was filtered out once again.

The filtrate was concentrated under reduced pressure (18 mm./40-45°C.) to about 150 ml. The concentrate was allowed to stand overnight in a refrigerator for the crystallisation of glutamic acid hydrochloride. Next day, the crystals formed were removed by filtration. The mother liquor was further concentrated to about 75 ml. and cooled to get an additional crop of the crystals. The two lots of the crystals were combined and recrystallised from hydrochloric acid solution. The final crystals were dried and weighed.

(*ii*) *Glutamic Acid.*—The crystals of glutamic acid hydrochloride were dissolved in a minimum quantity of hot distilled water. The pH of the solution was brought to 3.2 by the addition of sodium carbonate solution. The final solution was kept aside for 72 hours in a refrigerator for the crystallisation of glutamic acid. The crystals of glutamic acid formed in the solution were subsequently filtered. These crystals were washed with ice cold water until free from sodium chloride. The mother liquor and the washings were combined and concentrated to obtain an additional yield of glutamic acid. The two crops of crystals were combined, dried and weighed.

(*iii*) *Monosodium Glutamate.*—The glutamic acid thus obtained was dissolved in water. The solution was brought to a pH of 5.4 to 6.2 by the addition of sodium carbonate solution. The resultant solution was concentrated under reduced pressure to obtain monosodium glutamate. The concentrate on cooling gave monosodium glutamate in the powder form. This product was filtered, dried and weighed.

TABLE 3.—YIELD OF GLUTAMIC ACID HYDROCHLORIDE, GLUTAMIC ACID AND MONOSODIUM GLUTAMATE UNDER DIFFERENT CONDITIONS.

Oil-cake	Time	Sp. gr of acid	% of glutamic acid hydrochloride	% of glutamic acid	% of monosodium glutamate
Mustard					
(1)	6 hrs.	1.18	10.6	6.5	8.0
(2)	12 „	1.14	11.0	6.8	8.62
Linseed	12 „	1.14	6.11	3.7	4.37
Cotton	12 „	1.14	5.44	3.5	4.4

Discussion

The yeilds of glutamic acid and its products obtained have been summarized in Table 3. In this table are also indicated various conditions of hydrolysis of the oil cakes. It is noted that maximum yields of the amino acid and its products are obtained when conditions outlined in the methods are closely followed.

Production of glutamic acid invariably begins with hydrolysis of proteinous materials containing the acid. Hydrolysis can be brought about either enzymatically or with an acid or an alkali. At present, acid and alkali hydrolyses are largely employed. The acid hydrolysis is generally preferred and hydrochloric acid[4] is probably the best hydrolyzing agent. It is especially employed in the production of glutamic acid to be used for food purposes.

Protein hydrolysates made with sulphuric acid[5] as the hydrolyzing agent are not generally acceptable for food purposes, principally because of their inferior taste and high cost of production.

Protein hydrolysis in an alkaline solution[6] results in the formation of racemic glutamic acid. Alkaline hydrolysis is, nevertheless, used satisfactorily in the commercial manufacture of monosodium glutamate from Steffen's waste.

An excess of hydrochloric acid for hydrolysis should be avoided. The use of too much of the acid results in the formation of excessive quantities of sodium chloride which probably exerts an influence on the hygroscopicity of the final product in the powder form.[7]

In the present investigations it was found that hydrochloric acid of 1.03-1.14 specific gravity gives the best yield when the hydrolysis is conducted for 12 hours. Higher concentrations of the acid become troublesome during concentration and filtration of the hydrolysates.

During concentration of the hydrolysate under reduced pressure the distillate gives intenser meaty odour than that of the residual solution or even the crystals of monosodium glutamate. This odour might be due to amino acids other than glutamic acid present in the distillate. The hydrolysate which is always coloured, can be decolourised by the treatment with activated carbon. However, on concentration a slight colouration returns.

Conclusion

Glutamic acid, glutamic acid hydrochloride and monosodium glutamate can be produced with maximum yield from rapeseed, cottonseed and linseed oil cakes. The methods employed for the production of these materials have been so worked out as to make use of chemicals which are mostly available in Pakistan. The techniques of the production are quite easy and do not involve complicated procedure and equipment. With the production of essential amino acids, particularly gultamic acid from oil cakes another waste of Pakistan, rich in proteins, can thus be effectively utilised.

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SHARK MEAT FLOUR

S. Abdul Haq and S. Mahdihassan

Biochemical Research Division, Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi

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Shark meat minced, washed and treated with soya-bean powder, as a source of urease, gives urea free meat. It is then dried in a hot air blower and subjected to extraction with solvents to remove the incorporated fat. Finally it is dried in a hot air blower. Such a processed meat flour was free from fishy odour and had a protein utilization value of 61.

The importance of protein as a food constituent is universally recognized. However the superiority of fish protein over that of vegetable protein needs a word of explanation. It has been observed[1] that chickens fed on a diet consisting of 2-5% of fish protein grow better than those on a diet composed wholly of vegetable food. Thus the addition of about 5% of fish protein to their feed increased the growth rate of chickens in the first year by about 7% and decreased the feeding requirements by about 20%. According to Slovtzov[2] fish protein is equal to that of beef. Moreover when fish is substituted for beef the nitrogen is utilized better, resulting in a decreased excretion of uric acid in the urine. Further, fish meat produces a good influence on the assimilation of magnesium and phosphorus. Unfortunately the fishy odour is not usually liked and limits the use of fish as food. It is estimated[1] that about 2.7 million metric tons, i.e. about 13% of world catch of marine fishes, go to manufacture fish meal and oil. According to Dr. M.R. Qureshi, Director of Fisheries, Government of Pakistan, the landing at Karachi and the local consumption of all fishes for 1957 were as given in Table 1.

It is evident from Table 1 that only 1/6th of the total catch is consumed fresh and the rest, along with the total catch of sharks, generally goes to the curing yards for being salted and dried which is partly transported inland but mostly exported to foreign countries. It therefore seemed worthwhile to preserve such meat to its best advantage. Of all fishes shark meat deserved the most consideration. As compared with other fishes, shark flesh contains urea and on account of it receives a differential treatment. Whereas all other fishes are salted and dried, shark meat is first cut into slices and then thrown into pits with layers of salt in between and allowed to putrify. When most urea has been decomposed by bacteria, it is taken out and dried in the sun. Such urea-free salted shark meat however has a very unpleasant odour and is exported usually to Ceylon where apparently its putrid odour is no disqualification.

In order that shark meat may be generally used it had to be processed differently. The present trend is to make fish meat flour which can be used to fortify food stuffs and increase their portein value. The form in which fish meat is finally consumed, and its acceptability are the two factors which have been kept in mind during the experiments that are being described.

Experimental

Fresh sharks were procured from the market. The flesh together with the cartiledge was minced in a mincing machine washed rapidly with three changes of fresh water, and treated with a crude solution of urease. This consisted of 1% soya-bean powder which was added to the last washing

Table 1

No.	Names	Production in metric tons	Marketed fresh in metric tons
1.	Salmon and allied fishes	513	283
2.	Teleostean flat fishes	254	204
3.	Tunas, true mackerals and similar species	6337	1200
4.	Herrings, sardines etc.	6591	1000
5.	Other marine teleosteans	4050	1350
6.	Perches, croackers and other species	15345	3000
7.	Elasmobranchii	10000	—
	Total =	43090	7037

and the mixture was warmed to 40° C. to increase the activity of the urease. Finally the meat was tested for urea. After the complete removal of urea the meat was separated from most of its water in a basket type centrifuge without trying to remove soya-bean powder which could only enrich the protein. The meat was then dried at 50-55° C. in a hot air blowing oven. The dried material was powdered in a grinding machine so as to pass through a 30 mesh sieve. The shark contains only cartiledge which was also powdered along with the meat.

On drying, the loss by moisture was 65% but still left 10% of it. In this condition the fat was 4.0% or 1.0% of fresh weight. The dried powder with fat was transferred to a steam jacketed deodorising machine, which was provided with an electric stirrer, a suitable condenser and a thermometer for controlling temperature. "Solvent oil" (60-120°C. B.P. Burmah Shell) was added in the ratio of 1:6 w/v, on the dry weight basis. Extraction was allowed to continue for 3 hours with constant stirring at 65° C. The solvent oil was removed by filtering and then again the same quantity of fresh solvent oil was added and the pH of the mixture adjusted to 8.0 with a 6% ethanolic sodium hydroxide solution. The mixture was then refluxed for another 3 hours with constant stirring with the same pH and temperature. After 3 hours the mixture was removed from the solvent extraction vessel and transferred to a big pan. The excess alkali was neutralised with hydrochloric acid added dropwise. The mixture was allowed to stand for some time. The supernatant liquid was decanted off. The residue was filtered and the solvent oil from the wet residue was replaced by shaking it twice with petroleum ether which was used as 1:3 on the basis of the dry weight w/v. It was decanted and the meat further pressed and finally kept in a vacuum oven at 50° C. until the traces of petroleum ether were removed. After complete removal of petroleum ether the product was found free from its fishy odour. The deodorised product was found to contain 85% protein as determined by nitrogen estimation.

Details.—Experiments were conducted to determine the minimum period of treatment, using samples of 25 g. with 150 ml. solvent oil, at pH 8 and a temperature of 65°C. Samples collected at hourly intervals showed that 6 hours were required to satisfactorily deodorize shark meat. Next was required to determine the best ratio between the dry meat powder and the solvent which was to deodorize it. The satisfactory ratio proved to be 1:6, details being given in the Table 2.

The total recovery of the solvent oil was 85-90% and of petroleum ether 90%.

TABLE 2

S. No.	Wt. of sample	Quantity of sol. oil	Ratio	Deodorization
1.	25 g.	75 ml.	1:3	Not satisfactory
2.	25 g.	150 ml.	1:6	Satisfactory

The digestibility of the processed meat flour was found to be 99. Its protein utilization value was 61. These findings were carried out in Europe and we are obliged to Dr. van Veen of F.A.O., Rome, for kindly helping us in obtaining these data. The formula according to which digestibility and protein utilization value are calculated are given in an article by Griswald[3] and are reproduced in the following. Percentage digestibility is :

$$\frac{\text{Food N}-(\text{fecal N}-\text{metabolic N})}{\text{Food N}} \times 100$$

where metabolic nitrogen is fecal nitrogen on a nitrogen free diet. Biologic value or percentage of absorbed protein that is retained equals:

$$\frac{\text{food N}-(\text{fecal N}-\text{metabolic N})-(\text{Urinary N}-\text{endogenous N})}{\text{food N}-(\text{fecal N}-\text{metabolic N})} \times 100$$

where endogenous nitrogen is total urinary nitrogen on a nitrogen free diet.

The shark meat powder as determined here contained 800 mg. phosphorus and 300 mg. calcium per 100 g. of sample. On hydrolysis with papain the deodorized protein was found to contain appreciable amounts of the essential amino acids, amongst which, methonine, lysine and tryptophane may be especially mentioned. Their identification was carried out by paper chromatography using butanol, acetic acid and water as solvents. Solution of ninhydrin in butanol 0.2% was used as the spray reagent.

Discussion

Various methods have been tried by other workers to deodorize fish meat, including that of shark. There is a fermentation process[4] where the flesh is fermented in the presence of defatted butter milk. The method is found to be particularly suited to fishes, where the body oil is very low as is the case with the shark. Fermentation is claimed to result in almost complete removal of urea and partial deodorization.

South African scientists[5] have developed a process in which fish flour is treated with a mixture of ethyl alcohol and ethyl acetate in the ratio of 9:1. Five or more extractions were suggested, until the solvent appeared colourless. The processed flour was dried under vacuum to give a product free from fishy odour.

Iodine was also used to deodorize fish meat. Saturated iodine solution (4.2 ml.) in ethyl alcohol was mixed with glacial acetic acid (10.6 g.) and the solution diluted to 300 ml. with water and added to 1 kg. dried fish meat. The mixture was kept overnight in a sealed container and then dried to obtain a product claimed to be free from its odour.[6] In another effort by the Japanese scientists, raw whale meat was immersed, for 2 hours, in a solution composed of 11.5% $CaC0_3$, NaH_2PO_4 and KNO_3, and later on, for 1 hour in an acetic acid -tartaric acid solution of pH 5.0—6.0, to give an odourless product.[7]

In an Indian method,[8] alkali digestion was employed for deodorization of fresh meat which was first digested with 1% glacial acetic acid; this softens the meat and facilitates further operations. After separating water and removing traces of acid, the meat was refluxed with a suitable quantity of ethanol for 7 to 8 hours at 65-70°C. After necessary drying, the residue was digested with dilute sodium hydroxide. This treatment resulted in a fairly good solution of the meat. After filtering through a cotton plug, the filtrate was neutralized by adding dilute acetic acid and the protein precipitated and dried. The preparation obtained by this method was claimed to be almost free from any smell.

Several experiments were conducted by us to remove the fishy odour from shark meat by means of solvents like benzene, hexane, petroleum ether, "aviation spirit," etc., but in each case the odour returned on storing the samples. The only common solvent which proved successful was ethyl alcohol. The results obtained by ethyl alcohol and solvent oil, the latter being used in the present experiments, were almost the same. So far as the question of cost is concerned, solvent oil is far cheaper than alcohol and it is easily available in the local market. Solvent oil is Rs. 1/8/- and alcohol Rs. 3/8/- per gallon. The loss in the recovery of the solvent oil at the end of the process, was only 10%. The oil can be used again after freeing it from all odoriferous matter by treating the solvent with an oxidizing agent, e.g., $KMnO_4$ and distilling it, first on water bath at 80° C. and finally on an electric heater at 120° C. 2-3% of moisture was found in the final deodorized product. For the gift of Griswald's publication our thanks are due to Asia Foundation and particularly to Mr. Banningan, its present Representative.

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INSECTICIDAL EFFECTS OF SHARIGOL AND PYRETHRUM COMBINATION ON HOUSEFLY

M. S. Quraishi, A. Aziz Khan and A.B.M.L. Kabir

Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi

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In a process worked out by Siddiqui et al.[1,2] for the desulphurisation of coal and the simultaneous recovery of depolymerised coal resin by treating pulverised coal at sub-carbonization temperatures with super-heated steam, volatile sulphur-containing liquids were obtained as by-products. Pesticidal properties of these products which have been provisionally named as Makerol, Sharigol and Jhimpirol have already been reported.[3] The present paper deals with the synergistic action of Sharigol on pyrethrum.

As already mentioned, Sharigol is obtained as a by-product in the superheated steam treatment of coal from Sharig in West Pakistan.

The experiments were conducted on 3- to 5-day old flies bred under uniform conditions in the laboratory. The test chambers used were 2-foot cubes with port holes on three sides. The fourth side was fitted with a tight fitting sliding glass. Forty, 50 or 100 flies were released in the chamber and 0.4 ml. of the formulation was sprayed through the holes by means of a de Vilbis sprayer.

Through the glass side, the knock-downs (K.D.) were observed. Time taken for 50% and 75% knock-down were noted. After 15 minute exposure, the flies were sucked by means of an aspirator. These flies were then kept under observation for 24 hours and the number of dead flies was noted.

Experiments were done using 0.1% pyrethrins

Table 1.—The Results of the Experiments with Formulation A.

Experiment number	Number of flies used	Time (in minutes) for 50% K.D.	Time (in minutes) for 75% K.B.	K.D. in 15 minutes	Number of flies dead after 24 hours
1	2	3	4	5	6
1	40	2.2	4.3	40	38
2	40	2.4	5.4	40	36
3	40	3.1	4.6	40	38
4	40	4.0	5.2	40	35
5	40	2.0	4.0	40	36
6	40	2.5	4.2	40	38
7	40	2.2	4.4	40	37
8	40	2.1	4.2	40	38
9	40	4.2	6.1	38	35
10	40	3.5	5.6	38	36
11	40	4.0	6.2	37	34
12	40	4.1	6.0	36	35
13	40	3.2	5.2	40	32
14	40	4.6	6.0	38	24
15	40	4.7	6.5	37	22
16	40	2.7	5.2	37	38
17	40	3.3	4.2	36	22
18	40	3.0	5.0	36	28
19	40	4.0	5.6	37	26
20	40	3.5	4.5	36	21
21	100	3.2	4.4	90	62
22	100	3.9	5.2	95	63
23	100	3.6	4.8	98	58
24	100	3.0	4.0	94	62
25	100	3.2	4.9	96	67
Total	1300	82.2	125.7	1248	961
Mean		3.29	5.03		

in kerosene (formulation A) and 0.01% pyrethrins in kerosene plus 2% Sharigol (formulation B).

The experiments with formulations A and B were conducted simultaneously as far as practicable, otherwise they were conducted within as short an interval between them as possible, usually not more than twenty-four hours.

Twenty-five experiments were conducted using formulation A and 38 using formulation B. The results obtained are summarised in Tables 1 and 2, respectively.

Results

The statistical analysis was done by using Fisher's 't'. The calculated value of 't' is in the insignificant range, and the test thus provides no evidence against the hypothesis as regards 50% knock-down of flies.

Considering similar hypothesis for 75% knock-down of flies in column 4 of Tables 1 and 2 and using the previous formula, the value of t comes to 0.403. Here also, the value of t being insignificant, formulations A and B have got the same insecticidal effect as regards 75% knock-down of flies.

We now consider the knock-down in 15 minutes and test the hypothesis that the two formulations are similar with respect to the proportion of knock-down in 15 minutes.

TABLE 2.—THE RESULTS OF THE EXPERIMENTS WITH FORMULTION B.

Experiment number	Number of flies used	Time (in minutes) for 50% K. D.	Time (in minutes) for 75% K. D.	K.D. in 15 minutes	Number of flies dead after 24 hours
1	2	3	4	5	6
1	40	2.8	4.9	40	18
2	40	3.5	5.0	40	32
3	40	3.9	4.6	40	32
4	40	4.0	5.8	40	25
5	40	3.7	6.0	40	26
6	40	3.0	4.2	40	28
7	40	4.1	6.1	40	18
8	40	3.6	5.7	40	22
9	40	3.7	6.0	39	28
10	40	3.0	5.0	38	25
11	40	2.8	5.8	38	26
12	40	2.8	4.8	38	22
13	40	3.0	5.1	39	18
14	40	2.0	5.0	40	18
15	40	3.0	6.0	38	22
16	40	3.0	5.0	38	24
17	40	2.1	6.5	39	23
18	40	2.2	3.1	40	25
19	40	2.2	4.0	40	26
20	40	3.6	6.1	38	22
21	40	3.9	4.9	36	25
22	100	3.1	5.3	96	93
23	100	3.2	5.2	98	97
24	100	4.0	5.3	96	96
25	100	4.2	5.0	98	95
26	100	2.6	4.1	100	97
27	100	3.1	5.1	100	96
28	100	2.9	4.9	100	95
29	40	2.8	5.0	38	26
30	40	2.0	3.1	36	23
31	40	3.6	4.0	38	18
32	40	3.0	4.4	38	20
33	40	3.2	4.9	36	19
34	40	3.0	4.4	37	20
35	40	2.7	4.1	36	25
36	40	2.5	4.9	38	23
37	40	3.0	4.5	37	18
38	40	3.3	4.4	36	21
Total	1940	118.1	188.2	1879	1387
Mean	—	3.11	4.95	—	—

In this case, since in all 1300 flies were treated with formulation A, the number 1300 may be regarded as the first sample size (n_1). Similarly, 1940 is the sample size (n_2) with formulation B.

The proportion of knock-down with formulation A is

$$p_1 = \frac{1239}{1300} = 0.953$$

and that with formulation B is

$$p_2 = \frac{1879}{1940} = 0.968$$

Thus $p_1 \sim p_2 = 0.015$.

On the basis of the above hypothesis, we have as our estimate of the common value of the proportion of knock-down (p) in the population

$$p = \frac{n_1 p_1 + n_2 p_2}{n_1 + n_2} = 0.962$$

The variance of the difference of the proportions for the two samples is

$$e^2 = pq\left(\frac{1}{n_1} + \frac{1}{n_2}\right), \quad \text{where} \quad q = 1 - p$$

$$= 0.00004679$$

Hence $e = 0.007$

The observed difference being less than 3e is insignificant, and the test thus furnishes no evidence against the hypothesis.

Applying similar hypothesis and the same test for column 6, i.e. the number of dead flies after 24 hours, we get

$p_1 = 0.739$
$p_2 = 0.715$
$p_1 \sim p_2 = 0.024$
$p = 0.725$
$e = 0.016$

In this case too, the observed difference being less than 3e is insignificant, and therefore, none of the formulations can claim any superiority over each other as regards the proportion of dead flies after 24 hours.

To sum up, our analysis of the data shows that Sharigol, when used in conjunction with 0.01% pyrethrum, gives the same effect as 0.1% pyrethrum thus establishing that it activates pyrethrum in killing house flies.

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STUDIES ON THE SPORULATION OF MACROPHOMINA PHASEOLI (MAUBL.) ASHBY. CAUSING STEM-ROT DISEASE OF JUTE WITH REFERENCE TO THE POSSIBLE CAUSES OF OUTBREAK OF THE DISEASE IN NATURE

M. HUSSAIN* AND Q. A. AHMED

Jute Research Institute, Tejgaon, Dacca

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The present paper deals with the investigation of factors which might favour the spread of the stem rot, by far the most important disease of jute plant. Studies on temperature and humidity favouring the development of pycnidia on the affected plant and germination of pycnospores have been taken.

It is found that pycnidial pustules of *Macrophomina phaseoli* develop within a wide range of temperature (29-40°C.) and relatively narrow range of humidity (81-100%). Pycnospores on the other hand could germinate in even wider range of temperature (20-41° C.) the range of relative humidity is however, narrower (96-100%).

Introduction

Stem rot is by far the most important disease of jute plant. On the seedlings the disease manifests itself as dark lesions both on hypocotyl and on cotyledons. Throughout the growing period, due to secondary infection by air borne pycnospores the disease may appear on any part of a leaf showing buff coloured lesions. Later, the entire leaf including the mid-rib becomes affected. On the stems, the lesions generally start at the nodes coming in contact with a diseased leaf. These are small at the beginning , but later on may girdle the entire stem, causing the plant to die or a break to appear at the point of girdling (Plate 1).

The disease may spread from several sources as the organism can be seed-borne as well as soil-borne[8,14] through the diseased stubbles. Besides, there are innumerable collateral hosts[6,11,13,15] for this organism, from which the disease may easily spread through air-borne spores. Even with great care in eliminating the primary sources of inoculum, by sowing as far as possible healthy and treated seeds and also adopting crop rotation, there would always remain possibility of the presence of the organism on the seedlings through a certain percentage of infected seeds and from the collateral hosts, which are so many that some one always would be found growing in the neighbouring fields. This spread could be checked beforehand by the application of fungicides at proper moment if all the predisposing factors for such spread are well known.

The purpose of the present investigation is to study the various possible factors which might favour the spread of the disease. For this, studies on the factors such as temperature and humidity favouring the development of pycnidia on the affected plants and germination of pycnospores have been undertaken.

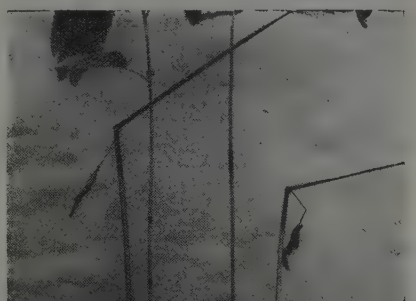

Plate 1.—Jute plants showing break at stem-rot affected region.

Experiments and Results

Effect of Temperature and Humidity on Sporulation—To obtain plants with stem-rot lesion, a number of plants, 21 days old was artificially inoculated with mycelial fragments of *M. phaseoli* grown for 48 hours exposure to 32°C. within a humid chamber and then were removed beside the glass windows inside the laboratory for 4 days.

From each of the affected plants (Plate 2) diseased portion showing black lesion was cut out keeping some healthy tissues at both ends of the stem piece. The size of the individual pieces was about 1.5-2 inches in length.

To find out the effect of temperature on the

*Now at Texas A. & M. College, U.S.A.

Plate 2.—Jute plants showing stem-rot lesion at the region of artificial inoculation.

production of pycnidia and pycnospores four pieces of the affected stem were placed within a sterilized petri dish having two No. 1 Whatman sterilised filter papers soaked with 10 ml. sterilised redistilled water so that maximum humidity prevails within the dishes. The stem pieces rested on two glass rods to avoid direct contact with soaked filter paper. Three replicates of such dishes were kept under each of the 6 temperatures, such as 2°, 25, 30, 35, 40 and 45°C. maintained within the respective incubators. Three further replicates were kept at room temperature, which fluctuated between 29-37° C. during the period (7 days) under experiment (Table 1).

TABLE 1.—EFFECT OF TEMPERATURE ON THE PYCNIDIAL DEVELOPMENT OF M. PHASEOLI.

No. of cuttings	Temp. in °C	No. of hours for pycnidial development.	Average No of pycnidia per field (1.77 sq. mm.)
12	20	0	0
12	25	0	0
12	29-37	56	16
12	30	96	7
12	35	92	11
12	40	100	3
12	45	0	0

Results show that no pycnidia developed at 25°C. or below. At room temperature which fluctuated between 29-37°C, number of hours required for pycnidial development was much less than those under constant temperature of 30,35 and 40°C. Among the constant temperatures, 35°C. required minimum time for pycnidial formation than either of 30 or 40°C. The number of pycnidia developed per microscopic field was also higher at room temperature.

A second experiment was conducted similarly as above inside sterilised dishes each having two No. 1 Whatman filter papers but this time affected stem cuttings were exposed to a large number of combinations of different relative humidity and temperature. The relative humidity at which stem cuttings were exposed were 65, 81, 88, 92, 96, and 100%. These were prepared by using a volume of 10 ml. redistilled water and respective aqueous saturated solutions of the different chemicals[1] (Table 2) to each of the dishes prepared as above.

TABLE 2.—COMPOSITION AND CONCENTRATION OF SOLUTION USED FOR MAINTAINING SPECIFIC HUMIDITY WITHIN AIR-TIGHT PETRI DISHES.

Materials used	Concentration of solution.	Theoretical R.H. %.
Redistilled water		100
$H_2C_2O_4.2H_2O$	Saturated aq.	96
K_2HPO_4 ..	„	92
K_2CrO_4 ..	„	88
$(NH_4)_2SO_4$ ..	„	81
$Mg(C_2H_3O_2)_2.4H_2O$	„	65

In all cases two replicates were used and incubated up to 7 days. Observations were taken periodically and time taken for pycnidial development under each treatment was noted (Table 3).

As the development of pycnidia at 20 and 25°C. was not observed in the previous experiment (Table 1), these two temperatures were not included in this experiment. It was presumed that the exactness of the relative humidity mentioned would suffer as the same saturated solutions were used under different temperatures. But this would be the case with all the relative humidity tested under each temperature.

Pycnidia were found to develop within a wide range of temperature and relative humidity. Fluctuating temperature from 29°C. to 37°C. (room temperature) gave the best result both from the

TABLE 3.—EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY UPON THE DEVELOPMENT OF PYCNIDIA OF M. PHASEOLI.

No. of cuttings per treatment	Temp. in °C.	Percentage of R. H.	Hours taken for pycnidial development	Average No. of pycnidia per microscopic field (1.77 sq. mm.)
4	30	100	96	7
,,	,,	96	100	6
,,	,,	92	104	5
,,	,,	88	116	4
,,	,,	81	132	3
,,	,,	65	0	0
4	35	100	88	8
,,	,,	96	92	12
,,	,,	92	96	10
,,	,,	88	96	10
,,	,,	81	100	8
,,	,,	65	0	0
4	40	100	112	3
,,	,,	96	132	4
,,	,,	92	116	4
,,	,,	88	112	4
,,	,,	81	112	4
,,	,,	65	0	0
4	29-37	100	56	16
,,	,,	96	60	15
,,	,,	92	60	14
,,	,,	88	60	14
,,	,,	81	60	9
,,	,,	65	0	0

stand point of earlier formation as well as greater frequency per unit area. At 65% relative humidity under any of the temperatures tested, no pycnidial appearance was noted.

Effect of Temperature and Relative Humidity on Germination of Pycnospores.—Requirements of favourable temperature and humidity for germination and infection have been studied with spores of fungal pathogens such as uredospores of *P. graminis*,[12] oidia of *Erysiphe polygoni* [10] and with spores of other organisms by different workers. [2, 3, 4, 5, 9].

With the present organism, the first experiment undertaken here was the effect of different temperatures on the germination of pycnospores in hanging drop culture. The temperatures at which the spores were tested for germination were 5, 10, 15, 20, 22 27, 30, 33, 36, 40, 41 and 45°C. regulated by different incubators.

For preparation of hanging drop culture a drop of sterilized water was taken on a clean cover glass and 2-3 pycnidia from affected stem were gently pressed to liberate the spores in water. The cover glass was inverted carefully without disturbing the water drop and placed over a glass ring already stuck on a slide with Vaseline. The cover slip was similarly attached by means of Vaseline, smeared at three places on the upper surface of the ring. The hanging drop culture thus prepared was placed within a pair of petri dishes having two saturated filter papers with redistilled water. Four replicates of these hanging drop cultures were put up for each temperature. The hanging drop cultures within the petri dishes were then placed inside respective incubators. Observation under microscope was carried at an interval of 30 minutes up to a period of 6 hrs. After 6 hrs. of incubation the cover slips containing germinating spores were lifted from the ring and mounted on a drop of cotton blue glycerine over a clean microscope slide. Each slide was examined under microscope at different fields. Average of 10 readings of spores counted and number germinated per microscopic field has been tabulated (Table 4) for each temperature.

TABLE 4.—EFFECT OF DIFFERENT TEMPERATURES ON THE GERMINATION OF SPORES AND THE LENGTH OF GERM TUBE IN HANGING DROP CULTURE.

Temperature in °C.	No. of spores counted	No. of spores germinated	Percentage of germination	Time taken for germination of 25% spores (in hrs.)
5	312	0	0	0
10	688	0	0	0
15	712	0	0	0
20	318	133	42.05	—
22	722	329	45.43	4.00
27	705	467	66.25	3.50
30	690	521	75.50	3.00
33	700	525	75.00	3.00
36	688	436	63.37	3.50
40	334	107	32.00	—
41	743	117	15.74	5.50
45	697	0	0	0

The range of temperature from 27° to 36°C. appeared suitable for good germination of pycnospores. No germination was observed at 5, 10 and 45°C.

So far the experiment on germination of spores was done in water suspension at different temperatures. It was intended here to find out the effect of different relative humidity on rates of germination at a constant temperature. The germination tests were carried out with 100, 99.04, 98.65, 98.03, 96 and 93% relative humidity at 20°C.[1'9].

The humidity chambers were prepared out of fruit jars of 1,000 ml. capacity having their glass lids

grounded, so that airtight conditions could be maintained. Before pouring the respective chemicals in solutions and at saturated condition, the glass jars were thoroughly cleaned, dried and rinsed with redistilled water.

Spores were taken out from pycnidia with the help of a clean and sterilized sharp needle-head and streaked over clean and sterilised cover glass, that served as spore-carrier. Each carrier was then attached with paraffin to one end of a small glass rod. The other end of the rod was heated and embedded in paraffin inside one glass ring that had been sealed with paraffin to the inner part of a glass top of a humidity chamber. This top was then substituted for one on a chamber (Plate 3) kept inside the incubator. A set of two replications were made with each relative humidity and kept at a constant temperature of 20°C. within an incubator for 24 hours. One similar set of two replications from each were kept at room temperature (31-37°C.) and a third set was kept at constant temperature of 30°C. After 24 hours the cover glasses were detached from the glass rod, cleaned thoroughly at the point of attachment and last trace of wax was removed before mounting on a drop of cotton blue, put upon a clean slide. Percentage of germination of spores and length of germ tubes were noted in Table 5.

It appears from this experiment that atmospheric humidity up to 99.24% at 30°C. favoured only a small (11.24%) percentage of spores to germinate even in 24 hours duration, whereas in a fluctuating temperature of 31-37°C. the percentage of spore germination raised sharply (59%). This was found due to the condensation of water in droplets on the spore-carrier.

Observation on the Occurrence of Stem-rot Disease in Nature.—Regular observations on the occurrence of stem-rot disease in the experimental plots of Pakistan Central Jute Committee was carried out right from the 4th week of April, 1959 when seedlings in most of the fields were 7 days old. Stem-rot affected plants were frequently met with in the plots of 'district trial' as well as in the plots of 'method of sowing' experiment. These two experimental plots were kept under observation. From the first week of May till the 3rd week of June, although there was quite a large percentage of plants both in the 'district trial' and 'method of sowing' trial, were found affected with stem-rot disease, very few plants showed pycnidial pustules on the lesions unless one was completely dead due to heavy attack. Appearance of pycnidial pustules on leaves were seldom met with up to this time. On the June 3, 1959 all the affected plants from 'district trial' fields were rouged out and the fields were kept clean from any undergrowths. This was not done with the plots of the 'method of sowing' experiment. There were profuse undergrowths

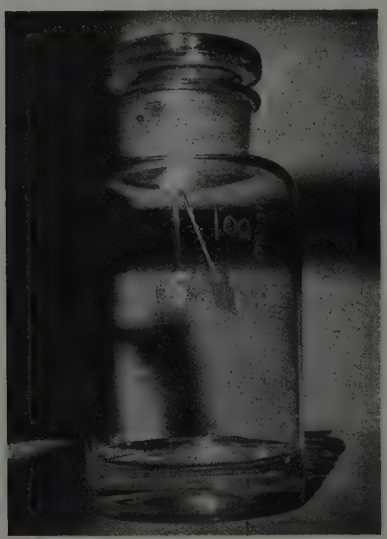

Plate 3.—Humidity chamber with three spore carriers hanging from the lower end of the glass stopper.

TABLE 5.—EFFECT OF RELATIVE HUMIDITY ON THE GERMINATION OF PYCNOSPORES AND DEVELOPMENT OF GERM TUBES.

Temp. °C.	Percentage of R. H.	No. of spores counted	No. of spores germinated	Percentage of germination	Average length of 10 germ tubes after 24 hours in microns
20	100	325	40	12.30	—
,,	99.24	301	21	7.0	104
,,	98.65	316	17	5.38	92
,,	98.03	324	13	4.01	78
,,	96.0	299	7	2.34	40
,,	93.0	307	0	0	0
30	100	320	91	28.44	—
,,	99.24	293	34	11.24	176
,,	98.65	322	29	9.00	149
,,	98.03	319	24	7.52	123
,,	96.00	319	12	3.76	66
,,	93.00	—	0	0	0
31-37	100	303	182	60.00	600
,,	99.24	310	183	59.00	600
,,	98.65	318	130	41.00	140
,,	98.03	309	92	29.76	125
,,	96.0	325	8	2.46	27
,,	93.0	216	0	0.00	0

of grasses together with the accumulation of leaves shed from the standing jute crop. Sporadic stem-rot affected plants were also present in the field. On the June 24, 1959 some leaves of scattered plants were found with the appearance of pycnidial pustules. By 27th of the month the disease appeared on the leaves in epidemic form. In some blocks 50% plants (Table 6) showed most of their lower leaves affected with stem-rot disease with characteristic pycnidial pustules. This was, however, not found with the jute plants of the 'district trial.'

TABLE 6.—PERCENTAGE OF PLANTS SHOWING PYCNIDIAL PUSTULES ON LEAVES.

Block No.	Total plants	No. of plants with leaf infection	Percentage of affected plants
1	813	241	29.64
2	778	198	25.45
3	490	285	58.16
4	1372	432	31.47
5	1421	392	27.58
6	1060	588	55.47
7	1307	405	31.06
8	443	221	50.00

The accumulation of leaf debris and dead affected plants amidst the undergrowths of grasses under the standing crop and the prevailing weather condition obviously influenced the sudden spread of the disease and the appearance of pycnidial pustules on leaves of the plants in the plots of the 'method of sowing' experiment during the 4th week of June. There was plenty of rainfall almost daily during a 12-day period excepting one day in the middle and consequently the field soil was more or less in saturated condition. The above period was followed by six rainless, warm and sultry nights having wind velocity from 1-8 (average 4.6) miles per hour.

Almost every day up to 8 o' clock in the morning during this week leaves of jute plants in the field were found to remain completely soaked with condensed water deposited from the exudation from the leaves. This condensed water on the surface of leaves which might have lasted more than 8 hours (from mid-night till 8 a.m.) would favour the germination of pycnospores[7] which might have been blown to the leaf surface earlier from the undergrowths of the standing crop, where dead plants and fallen leaves with pycnidial pustules were present.

The absence of pycnidial development during the second and third week of June (when almost every day, there was heavy rain) may be explained due to the sweeping down of the pycnospores from the leaves if there was any, deposited on the leaf surface by the air current. Same explanation might be cited for gradual disappearance and from further spread of the disease on leaves of the plants of the same field during the first week of July, 1959, when there was rainfall in almost every day thereby splashing the air-borne spores from leaves on earth or there might be some other explanation for the above observation which could not be put forward in the present work.

From the available data with Jute Research Institute for the preceding years, it was found that the appearance of pycnidial pustules on leaves of jute plant during 1958 was practically nil but in 1957 twice during the growing season, once during the fourth week of May and another time during the 3rd and 4th week of October there were abundant pycnidial pustules on leaves. From weather bulletin it was found that both the times pycnidial pustules appeared during the rainless days which were preceded by periods having heavy shower of rain. The wind velocity in the two periods respectively was from 6-9 (average 8.3) and 1-3 (average 1.5) miles per hour.

Discussion

Among the range of temperature used (Table 1 and Fig. 1) the constant temperature 20 and 25°C. did not favour the pycnidial development. Among the three other constant temperatures used, 35° C. favoured earlier development of pycnidia. This happened within 92-hour exposure of the affected stem pieces to 35°C. The fluctuating room temperature (29-37°C.), however, was found best both from the point of earlier development (56 hours) and frequencey of pycnidia per unit area. As regards effect of humidity on pycnidial development, their appearance was noted within 81 to 100% R. H. (Table 3 and Fig. 2).

Pycnospores were found to germinate from 20°C. to 41°C. in hanging drop culture (Table 4 and Fig. 1) the peak germination was found at 30°C. (75.50). The best range for germination was found between 27 and 36°C. A constant temperature of 30°C. relative humidity as high as 99.24% could not stimulate the germination of pycnospores beyond 11.24% (Table 5) which was very low when compared to that of hanging drop culture under the same temperature. At 100% relative humidity (Table 5) though germination rate accelerated somewhat more, it was still very low in comparison to that of hanging drop culture. Similar observation was also made by other workers,[4,13] with uredospores of *Puccinia glumarum* and *P. graminis tritici* and with many

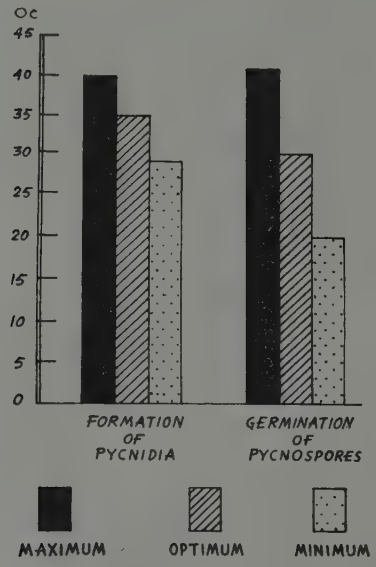

Fig. 1.—Range of temperature for pycnidial formation and germination of pycnospores.

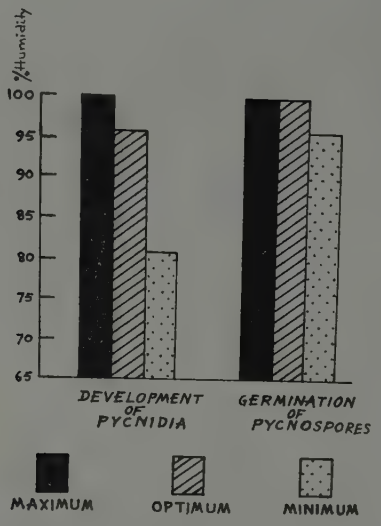

Fig. 2.—Range of relative humidity for pycnidial development and germination of pycnospores.

other pathogenic fungi. At fluctuating room temperature (31-37°C.), however, when there was condensation on spore-carriers kept at 99.24, 98.65 and 98.03% R.H. the germination rates increased decidedly more than those met with under similar relative humidity at a constant temperature of 30°C. (Table 5). At 30°C. constant temperature no condensation of water was noticed.

From the foregoing observations it appeared that pycnidial development could take place within wide range of temperature (29-40°C.) and atmospheric humidity (81-100%), whereas germination of pycnospores though could take place within wider range of temperature (20-41°C.), the range of favourable humidity was very narrow (Table 5 and Fig. 2). The germination was observed much better in presence of free water than at high atmospheric humidity of 99.24% or 100%.

In nature the spread of the disease in an epidemic form was noticed just after a few days when excessive condensation of free water on the surface of leaves occurred almost every day up to 8 o'clock in the morning. Six days before this happened there was heavy rain almost everyday for ten consecutive days. This created optimum condition for the development of pycnidia and pycnospores on the existing scattered diseased plants and on the littered leaf debris amidst the undergrowths of grasses at the foot of the standing crops. The source of inocula thus was ready in the field from which pycnospores were carried by air current to the leaf surface throughout the day and night. During the six rainless and sultry nights in the presence of condensed water on the leaves the germination of pycnospores might have followed by infection and penetration, resulting in epidemic condition in the field.

Summary

Pycnidial pustules of *Macrophomina phaseoli* was found to develop within a wide range of temperature, from 29-40°C. and within 81-100% relative humidity.

Pycnospores on the other hand though could germinate within even wider range of temperature (20-41°C.), the range of favourable atmospheric relative humidity was however found to be very narrow (96-100% R.H.). Most favourable range of temperature for germination was found to be from 27-36°C. At a constant temperature of 30°C. in humid atmosphere (100% R.H.) only 28.44% pycnospores showed their germination within 24 hours whereas in fluctuating temperature of 31-37°C. in similar condition when condensation on spore-carriers occurred about 60% pycnospores germinated. In free water on the other hand at constant temperature of 30°C. over 75% pycnos-

pores put up their germ-tubes.

The above findings corroborated the observation made in a jute field during the month of June, 1959 where a week before there were only a few scattered plants with pycnidial pustules on some of their leaves. In some blocks the disease spread to 50% plants or over following excessive condensation of free water on the surface of leaves which was noted almost every morning during 5 to 6 days time.

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SHORT COMMUNICATIONS

PHYSICO-CHEMICAL STUDIES ON THE ESSENTIAL OILS OF CITRUS FRUITS

SAMIULLAH

North Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Peshawar

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Essential oils from citrus peels are an important source of flavouring materials for all kinds of beverages, confectionaries and bakery products. In view of their varied uses, essential oils especially citrus are in a great demand in Pakistan. There are about 120 fruit preservation concerns in Pakistan which do not fully utilise the citrus peels for the manufacture of essential oils. The preparation of these oils is a problem of economic importance to the country. Experiments were, therefore, undertaken to prepare essential oils from citrus peels by various methods and to study its characteristics.

Experimental

Distillation is not a suitable method for the isolation of certain essential oils which undergo destruction on exposure to heat, light and water. In such cases one of the three methods, viz., expression, extraction by means of volatile solvents and absorption in purified fats, is employed. Sponge method[1] described as under was used in the experiments reported in this article.

The fruit was cut in half and the pulp was separated from the peel by means of a special form of spoon with a sharp edge. This was inserted at the junction of the white albedo and the juice sacs. By a quick turn of the wrist the pulp was extracted whole without any liberation of juice. The peel was freed of adhering pulp, dipped in water and allowed to stand for four hours. It was then pressed between sponges in a hand press. The pressure on the peels breaks the oil cells and the oil together with some water spurts out and is collected in an earthenware jar provided with a lip from which the oil is decanted. Absorption by sponge breaks any emulsion that may have been formed by the colloidal substance present in water and the decanted oil is almost clear. During these experiments the mixture collected was centrifuged and physico-chemical studies on the clear oil were carried out by adopting the methods recommended by Earnest Guenther.[2] The data is given in Table 1.

Discussion of Results

The data given in the table show that physical constants like specific gravity, optical rotation, refractive index, evaporation residue and solubility are almost identical with those reported by other workers with certain notable variations. It is known[2] that slight variations in physical properties are caused by the admixture of different species of plants. Aldehyde content of various essential oils except that of bitter orange oil falls within the range cited in the literature.[2,3] The yield of bitter and sweet orange oils from the local fruit is higher than that reported in the literature for foreign varieties.

Summary and Conclusion

One of the expression methods, viz. sponge method, has been found to be suitable for the preparation of citrus oils. It is felt that small medium size factories could profitably utilise this method for the preparation of citrus oils as a by-product of squash manufacture. The quality of the oil obtained by this method compares favourably with the standards cited in the literature.

Acknowledgement : The author is thankful to Col. M. K. Afridi for encouragement and facilities afforded for this work and to Dr. C. M. Ishaq for his guidance and suggestions.

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Physico-chemical studies on the essential oils of citrus fruits (Contd.)

TABLE 1.—CHARACTERISTICS OF CITRUS OILS.

Sr. No.	Name of essential oil	Yield %		Specific gravity at 25°C.		Optical rotation at 25°C.		Refractive index at 20°C.		Aldehyde content as decylaldehyde %		Evaporation residue %	Solubility at 20°C.	
		Obtained	Reported	Observed	Reported	Observed	Reported	Observed	Reported	Obtained	Reported	Obtained	Reported	Observed	Reported
1.	Bitter orange oil**	0.76 to 0.9	0.15* 0.33**	0.844	0.845-0.851	+99.9°	+91°0-+96°21'	1.4740	1.473°-1.4760	1.91	1	4.06	2.2-4.7	Soluble in 7-8 vols. of 90% alcohol.	Souble in 4 vos. & more of 95% alhcool.
2.	Sweet orange oil.**	0.531	0.3* 0.277-0.318**	0.842	0.842-0.846	+98°.69°	+96°-+99°	1.4735	1.4723-1.4742	2.1%	0.8-2.1	1.84	1.6-4.5	Incompletely sol. in 10 vols of 95% alcohol.	Incompletely sol. in 95% alcohol up to 10 vols.
3.	Lemon oil**	0.247	0.35* 0.265-0.3**	0.843	0.849-0.855	+78.18°	+57°-+65°36'	1.4744	1.4742-1.4755	5.206 (as citral)	3.7-5.0	1.86	1.5-2.2	Completely sol. in 0.5 to 1 vol. of 95% alcohol.	Soluble in 3 vols. of 95% alcohol and more.
4.	Grape fruit oil**	0.427	0.06* 0.07**	0.853	0.8508 0.8532	+86.63°	+91°45'-+94°36'	1.4740	1.4745-1.4778	1.4	1.4-1.6	3.5	5.0 to 8.0 %	Incompletely sol. in 10 vols. of 90% alcohol.	Incompletely sol. in 90% alcohol up to 10 vols. owing to separation of wax.

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STANDARDIZATION OF ACTIVE PRINCIPLES OF INDIGENOUS PHARMACOPOEIAL DRUGS FOUND IN WEST PAKISTAN

MUKHTAR AHMAD WAHID AND SAMIULLAH

North Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Peshawar

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Introduction

West Pakistan has an abundance of medicinal herbs [1-4] and their scientific utilization can play an important role in the development of local pharmaceutical industry. Exploitation of these drug resources would not only make us self-sufficient in respect of some of our requirements but might also help us to earn foreign exchange by exporting surplus quantities.[8] Some of these herbal drugs are of recognised therapeutic value and if they come up to the standards laid down by some pharmacopoeias they would be a valuable asset to the country both from the medicinal and economic points of view. Each pharmacopoeia describes a certain lower limit of the percentage of active principles in a drug and if that condition is fulfilled, the drug can be accepted as official. Since no systematic work has so far been done in this direction it was considered advantageous to estimate the active ingredients of some important local pharmacopoeial drugs and thus to standardize them.

Materials and Methods

Samples of crude drugs used in this work were collected from the Experimental Farm of the North Regional Laboratories or from different localities of the north-western region of West Pakistan. A few samples were also obtained from the local market. They were dried in the shade or in the sun as required and the percentage of active principles was calculated on the basis of air dried weight of the sample. Some drugs were freshly handled because of the volatile nature of their active constituents and the percentage was calculated on the basis of the weight of fresh sample. The values of active principles represent the average of at least three determinations in each case. All drugs were analysed according to the methods prescribed in the British Pharmacopoeia 1958 or the Indian Pharmaceutical Codex 1953 and the results have been shown in Tables 1 and 2.

Discussion

A perusal of Table 1 shows that above mentioned drugs are standard and can be used where such pharmacopoeial drugs are required except oil of chenopodium and oil of eucalyptus because they fall short of the pharmacopoeial requirements. Oil of chenopodium according to B.P.C. 1954 should contain 65% ascaridol while that from the local herb contains only 46.0% ascaridol. Since its anthelmintic activity is well established a higher dosage level (5-20 minims) can be tried.[9] Oil of eucalyptus can be used in perfumery or for mineral flotation.[9] Colchicum corm contains 0.26-0.27% of colchicine and is a very good substitute for *C. autumnale* L., an important drug of the British Pharmacopoeia 1958, and can be recognised by that pharmacopoeia.

TABLE 2.—PERCENTAGE OF ACTIVE PRINCIPLES OF INDIGENOUS DRUGS INCLUDED IN THE INDIAN PHARMACEUTICAL CODEX 1953.

Botanical name	Common name	Source	Percentage of active principles	Lower pharmacopoeial limit
Aconitum chasmanthum, Stapf ex Holmes.	Aconite	Wild	Root: 0.63-3.8 indaconitine	Not specified
Artemisia maritima, Linn.	Wormseed	Wild	Buds & leaves: 0.71-1.81 santonin	0.75%
Berberis lycium, Royle	Indian barberry	Wild	Root: 4.31 berberine.	1.00%
Colchicum luteum, Baker	Colchicum seed	Wild	Seed: 0.40-0.44 colchicine	0.3%
Datura fastuosa, L.	Datura	Cultivated	Leaves: 0.12-0.26 hyoscyamine	0.25%
D. stramonium, Linn.	Stramonium	Wild	Seed: 0.23-0.29 hyoscyamine	0.2%
Plantago ovata, Forsk	Isfaghul	Cultivated	Seed: 7.0-9.0 ml. absorbancy	10.0 ml.
Polygala chinensis, Linn.	Indian senega	Wild	Root: 1.97 saponin	Not specified
Valeriana wallichii, DC.	Indian valerian	Wild	Root: 0.25-0.89 percent v/w volatile oil	0.5%

From Table 2 it is evident that all drugs given in this table fulfil the standards and requirements as laid down by I.P.C. 1953 and can be used where such drugs are specified. Absorbancy test of *Plantago ovata* is, no doubt, a bit low and can be raised in value by improved methods of cultivation. It can be used even as it is, because its action on intestines in diarrhoea or dysentery is only mechanical due to some mucilage and not chemical.

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TABLE 1.—PERCENTAGE OF ACTIVE PRINCIPLES OF INDIGENOUS DRUGS INCLUDED IN THE BRITISH PHARMACOPOEIA 1958.

Botanical name	Common name	Source	% of active principles	Lower pharmacopoeial limit
Atropa acuminata, Royle ex Lindley	Indian belladonna	Cultivated	Leaves: 0.54 hyoscyamine Leaves: 0.30-0.66 hyoscyamine	0.40%
Carum carvi, L.	Caraway	Market	Fruit: 3.30-3.50 v/w volatile oil	3.5%
Chenopodium ambrosioides, L. Var. anthelmintic	American wormseed	Wild	Plant: 0.17-0.25 volatile oil containing 46.0 percent ascaridol	Oil containing 65% ascaridol
Cinnamomum camphora, (L.) Nees & Eberm	Camphor.	Cultivated	1. Fresh leaves-0.60 camphor 2. Fresh twigs: 0.20 camphor	Not specified
Citrus aurantium, L. var. bigaradia	Bitter orange peel[6]	,,	Fresh peel: 0.94 v/w volatile oil	2.5 percent in dried peel
C. medica, L. var. limon.	Lemon peel	,,	Fresh peel: 0.30 v/w volatile oil containing 5.2 percent citral	Oil containing 4.0% citral
Colchicum luteum, Baker. (substitute for *C. autumnale*, L.)	Colchicum	Wild.	Corn: 0.26-0.27 colchicine	0.25%
Datura stramonium, L.	Stramonium	Wild	Leaves: 0.23-0.32 hyoscyamine	0.25%
Dryopteris filixmas (L.) Schott.	Male fern[5]	Wild	Rhizome: 2.50-3.39 filicin	1.5%
Ephedra gerardiana, Wall	Ephedra	Wild	Shoots: 0.83-2.04 ephedrine	1.25% (B. P. C. 1954)
Eucalyptus globulus, Lab.	Eucalyptus	Cultivated	Fresh leaves: 0.50-0.83v/w volatile oil containing 40-45 cineol	Oil containing 70 % cineol
Hyoscyamus niger, L.[7]	Henbane	Wild Cultivated	Leaves: 0.035-0.058 hyoscyamine Leaves: 0.30-0.045 hyoscyamine.	0.05%
M. piperita, L.	Peppermint	Cultivated	Fresh leaves and flowering tops: 0.15 v/w volatile oil	Not specified
Papaver somniferum, Linn.	Poppy	Market	Opium: 7.96-10.55 morphine	9.5%
Podophyllum emodi, Wall	Indian podophyllum	Wild	Rhizome & roots (i) 11.50-15.56 podophyllin (ii) 3.19-4.10 podophyllotoxin	Not specified
Rheum emodi, Wall	Rhubarb	Wild	Root: 4.30 cathartic principles	Not specified

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NOTES & NEWS

Tercentenary of the Royal Society

The tercentenary of the Royal Society was celebrated from July 18-26, 1960. The programme marked out the historical importance of the event and gave a good opportunity to those who took part in it to become acquainted with the active research work in Great Britain.

The formal opening ceremony was performed on the afternoon of July 19 in the Royal Albert Hall by H.M. the Queen, the Patron of the Royal Society, who was accompanied by the Duke of Edinburgh and the King and Queen of Sweden. Sir Cyril Hinshelwood, the President of the Society, delivered the tercentenary address.

In honour of the occasion the Universities of Oxford, Cambridge and London held special honorary degree ceremonies to confer degrees on distinguished scientists attending the celebrations.

Formal participation in the full programme was limited to Fellows and Foreign Members of the Society and to those invited to represent national academies of science, selected universities throughout the world, and appropriate international scientific organizations.

At the Society's tercentenary conversazione, held in Burlington House on July 23, a series of exhibits giving a presentation of current research work were included in the programme of the evening, during which there were also on display the congratulatory addresses received by the Society on the occasion of its three-hundredth birthday.

The celebrations concluded with a great banquet on July 26 at Grosevenor House attended by some 2,000 participants, and their wives and guests of the Society. The toast of 'The Royal Society' was proposed by the Prime Minister, Mr Harold Macmillan, and replied to by the President, Sir Cyril Hinshelwood.

In the celebrations, Pakistan was represented by Dr. Salimuzzaman Siddiqui, Director, Pakistan Council of Scientific and Industrial Research, and Dr. Abdus Salam, Professor of Applied Mathematics at the Imperial College of Science and Technology, London.

On this occasion the following congratulatory messages were sent from Pakistan to the President of the Society.

Pakistan Council of Scientific and Industrial Research

"The President and Members of the Pakistan Council of Scientific and Industrial Research wish to convey to the Royal Society their rejoicing and congratulations on the historic occasion of its tercentenary celebrations. We deem it a privilege to have this opportunity of extending our felicitations to the oldest scientific society of the world, which pioneered the release of human intellect from its subjective moorings, and has been a source of inspiration over the centuries for the scientific endeavours of mankind. In humble recognition of its glorious services to science we wish to present through you Mr. President our sincerest greetings and salutations to the Society."

University of Punjab

"The Vice-Chancellor and the Syndicate of the University of the Punjab wish to present their congratulations to the Royal Society on its Tercentenary as the oldest and premier scientific body in the world. It gives us the deepest pleasure to present our salutations for the great services the Royal Society has done to Science during the three hundred years of its existence and we are confident that the Society will remain one of the leaders for scientific endeavour in the future."

Academy of Sciences of Pakistan

"The President and Fellows of the Academy of Sciences of Pakistan wish to present their congratulations to the Royal Society on its Tercentenary. We deem it a privilege to present our salutations to the premier scientific Society in the world. The history of science has quite largely been synonymous with the history of the Royal Society. We are confident that the Society will remain one of the leaders for scientific endeavour in the future. We send to the Fellows of the Society, through you Mr. President, our sincerest greetings."

University of Karachi

"The Vice-Chancellor and the Syndicate of

the University of Karachi wish to present their congratulations to the Royal Society on its Tercentenary as the oldest and premier scientific body in the world. It gives us the deepest pleasure to present our salutations for the great services the Royal Society has done to Science during the three hundred years of its existence and we are confident that the society will remain one of the leaders for scientific endeavour in the future."

Mineral Exploration Project

An operational agreement between the Government of Pakistan and the United Nations Technical Assistance Board may be signed within the next few days giving the go-ahead signal for the implementation of a UN-backed mineral exploration project.

Out of a UN-Special Project Fund allocation of 1.5 million dollars, a major sum may be utilized for drilling at Bogra in an attempt to locate coal at a shallow depth of two to three thousand feet.

Some foreign experts have already visited the coal deposit site at Bogra which seem to be an extention of the Indian coal belt. The coal found at Bogra is said to be of a very high quality. The problem now is to locate coal at a shallow depth. Samples so far obtained came out of a test well drilling at a depth of about 9,000 feet which may render mining operation to be uneconomic.

The United Nations will be sending out experts and may also arrange for drilling contracts to help implement the mineral exploration project.

Fifty-four Projects Completed by P.I.D.C.

The Pakistan Industrial Development Corporation has so far completed 54 projects in the country under its industrialization programme. The total amount invested in these completed projects comes to Rs. 108.23 crores of which Rs. 28.69 crores have been contributed by the Central Government and the balance through foreign aid.

Six projects were completed last year, while 5 are under execution. These include iron ore prospecting at Kalabagh, two natural gas fertiliser factories, extension of Mample-Leaf Cement Factory and peat development at Faridpur. The five projects under execution are estimated to cost Rs. 43.22 crores including Rs. 3 crores under foreign aid. These projects will be ready within 2 years.

During 1959-60, the PIDC-sponsored projects produced goods estimated at Rs. 60.29 crores which showed an increase of 24 per cent over the production of 1958-59, while their exports during the year were estimated at Rs. 23.39 crores or about 46 per cent higher than that in the previous year.

Life Span in Italy

In the course of the past hundred years the average life span in Italy has more than doubled itself, increasing from 32 years to 68 years, according to statistics made public in Rome. This rise in the average life owes greatly to four main factors, viz., general improvement in the standard of living, welfare measures taken by the Government, rise in literacy and better sanitary and hygienic conditions.

New Journal

An important new international scientific research journal, *Radiochemistry, U.S.S.R.*, has been introduced from 1960. It contains selected translated papers from the Soviet journal *Radiokhimiia*. Dr. A. G. Maddock of the University Chemical Laboratory, Cambridge, England, is the Translation Editor, and the journal is published twice a year by Pergamon Press Ltd. The annual subscription is £ 15 or $ 40.

The new journal includes publications on a variety of physical and inorganic topics related to radiochemistry. This English version is intended for the rapid provision of adequate abstracts and translations of the more important papers. The variety of papers selected will constitute an important addition to the literature of this branch of radiochemistry.

Clear Photograph of Sun Spot

It was announced by the U.S. National Science Foundation that Scientists have succeeded in clearly photographing the centre of a sun spot for the first time and they discovered that it contains small white dots, possibly rising gas which transmits heat.

A stratoscope photograph was previously taken of a sun spot on September 24, 1959 by the Princeton University astronomy team, in the last of a series of balloon-borne observations.

It was pointed out by the Foundation that the previous astronomical observations had hinted that dots existed, but clear photographs had never been taken before.

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